

Proceedings of the World Buiatrics Congress 2016



The
29th
Congress of the
World Association
for Buiatrics

THEME:

**Cattle Health -
Tomorrow's
Thinking Today**



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Dublin, Ireland, 2016

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Proceedings of the 29th Congress of the
World Association For Buiatrics, Dublin, Ireland 2016

CONGRESS PROCEEDINGS

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Through its commitment to the Science of Healthier Animals™, MSD Animal Health offers veterinarians, farmers, pet owners and governments one of the widest range of veterinary pharmaceuticals, vaccines and health management solutions and services. MSD Animal Health is dedicated to preserving and improving the health, well-being and performance of animals. It invests extensively in dynamic and comprehensive R&D resources and a modern, global supply chain. MSD Animal Health is present in more than 50 countries, while its products are available in some 150 markets. For more information, visit www.msd-animal-health.com or connect with us on LinkedIn.



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In Dairy, Elanco is committed to help veterinarians and dairy farmers with innovative solutions to improve cow's health and wellbeing with a focus on The Vital 90™ Days period, a time of great risk and vulnerability for every cow. Health is essential for a sustainable dairy production and herd health status is a key parameter to ensure productivity, efficiency and profitability of dairy farms. So there's nothing more frustrating than watching cows suffer from preventable disease. Elanco can provide tools & solutions to better manage Negative Energy Balance & Immune Suppression during The Vital 90™ Day.



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Merial is a world-leading, innovation-driven animal health company, providing a comprehensive range of products to enhance the health, well-being and performance of a wide range of animals. Merial employs around 6,200 people and operates in more than 150 countries. Merial is a Sanofi company.

Merial is a leader in vaccine technology, a pioneer in parasite treatment for animals and has a solid track record of best-in-class products. Its expertise is extensive in both veterinary pharmaceutical products and vaccines (biological) for companion and production animals.

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Animal Health Ireland

Animal Health Ireland is a not-for-profit, public-private partnership, tasked with controlling livestock diseases not subject to international regulation, with the objective of improving the profitability and sustainability of Irish farmers and the agri-food sector.



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Bimeda Animal Health

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Bio-X Diagnostics

Bio-X Diagnostics, ISO 9001:2008 certified is developing diagnostic reagents for diseases affecting production animals such as cattle, pigs, horses, small ruminants, trouts and carps. Thanks to the multi-pathogen tests that Bio-X Diagnostics has developed, a single sample can be tested for several pathogens at once.



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Biomed Diagnostics manufactures the Gold Standard InPouch TF Bovine, a test critical to diagnosis of Trichomoniasis among other diseases. The In Pouch format saves time, improves workflow and reduces sample contamination.



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Intracare is a dynamic company that develops sustainable solutions for the agriculture sector by reducing the amount of pathogens (disinfection), improving animal health (feed program), and developing non-antibiotic medicine.



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Vetoquinol

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Science For A
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Bayer Sponsor Symposium 120

Dynamics in animal health on beef and dairy cattle
Dr. Calvin W. Booker and Prof. Ynte H. Schukken



Boehringer Ingelheim Symposium 121

Make BVD history
Volker Moennig, Christian Guidarini and James A. Roth



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Elanco Immunity Side Symposium
Mr. Mike Steele



Merial Sponsor Symposium 123

Early BiRD gets the germs
David Homer, Sandro Cavirani and Claude Hamers



MSD Animal Health Sponsor Symposium 124

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Evolution of Bovine Surgery

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Tremendous advances have taken place in food animal practice, especially in the area of production medicine. However, anesthesia, analgesia, and surgery are still routinely performed on food animals and are primarily performed in the field on farms, ranches, and feedlots. The ability to diagnose and manage surgical problems is one basis for gaining the trust and confidence of a client. These procedures range from castration to laparotomy. Economic, welfare and other constraints often prevent cattle from being referred to surgical centers for specialized care. Difficult surgery can be performed on the farm and advances in surgical materials for the bovine patient make these procedures more approachable. Advancements in bovine surgery, such as minimally invasive surgery, have allowed improvements in bovine surgical care to advance despite economic limitations that are often present. Surgical management of disorders of ruminants continues to evolve with time. Improvements in chemical restraint, surgical technique, and pain management require the ruminant surgeon to remain vigilant in their professional development so that the client and patient can be afforded the best care possible. Constraints experienced by the ruminant surgeon often involve economics, regulatory prohibitions, and dogma. Limitations of ruminant surgery occur because of patient size, weight and mentation; surgeon knowledge, training or skills; and because the client may lack the ability to fully comply with needs during the convalescent period. Dogma, knowledge, skills, and training all can be changed through continuing education, research, and innovation. It has been my life-long desire to enable ruminant surgeons to advance their knowledge of the science and that this will result in personal development in the art of surgical practice. Surgical correction of problems nearly always requires anesthesia and advancements in the clinical practice of surgery often follow improvements in anesthesia. A critical component of peri-operative management of surgical patients includes pain management. Recent advances in analgesic therapy have centered around pain management. I endorse alleviation of pain whenever possible. Recent introduction of analgesic protocols allow for these procedures to be done in many situations and may prevent the loss of valued livestock. Regulatory issues regarding the potential for drug residue adulteration of food products must be heeded to ensure that we do not compromise the client or the consumer.

Pain Management

Management of pain continues to be an important consideration in livestock on which surgical procedures are performed.^{1, 2} In these animals, a balance must be achieved between the need to mitigate discomfort and the economic constraints of the production enterprise. Moral and ethical dilemmas have increased amongst consumers and these concerns have stimulated interest to re-examine the methods used to achieve the shared goals of humane production of safe, affordable animal products for human consumption. Administration of drugs to mitigate pain is variable among veterinarians. In field settings, surgical procedures are performed both on an elective and emergency basis. These surgeries are expected to cause variable degrees of pain or distress. Pain and the biological responses to it are part of a highly integrated multidimensional system that cause animals to react to protect themselves from the noxious stimulus.³⁻⁵ The magnitude of surgical pain is influenced by the procedure, the methods used, and the experience and skill of the practitioner.^{1, 2, 6} Some strategies to inhibit or minimize pain before it occurs (e.g. pre-emptive) are obvious: local anesthesia, general anesthesia, sedation, and tranquilization. Pre-emptive therapy should be done with the intention of controlling pain throughout the

stimulus which may last for hours, days, or weeks. A variety of anti-inflammatory drugs are available for clinical use (Table 1).^{1,2,6} Steroids and non-steroidal anti-inflammatory drugs may have benefits to the patient such as mitigation of pain, lessening of swelling, diminishing inflammation at the incision site and/or damaged tissues, and more rapid patient recovery after the procedure. Non-steroidal Anti-inflammatory Drugs (NSAID's) inhibit cyclooxygenase enzymes (COX).^{1, 2, 6} COX acts on arachadonic acids to liberate prostaglandins and other mediators of inflammation. Multiple isoforms of COX enzyme exist with COX-1 recognized as primarily involved with normal homeostatic mechanisms and COX-2 as an induced enzyme in response to injury. COX inhibitors prevent production of these factors. Non-specific COX inhibitors include aspirin, flunixin, and phenylbutazone. More selective COX-2 inhibitors are rapidly evolving and include etodolac, carprofen, and meloxicam. NSAIDs have differential activity because of the presence of variable receptors and variable drug effects. Our clinical experience suggests that although these drugs may be safer for long-term use (e.g. COX-2 inhibition is less likely to interfere with homeostasis of abomasal mucosa or renal perfusion), COX-2 inhibitors provide less potent analgesia.

Much of the pain research performed has shown benefits of pre-emptive analgesia. There is a less marked effect of administration of analgesic medication after the noxious stimulus has become established. In a study where flunixin meglumine was administered before laparotomy for correction of abomasal displacement, cows receiving flunixin (2.2 mg/kg IV) had significantly greater rumen contracts during the first 24 hours after surgery as compared with control cows.⁷ This administration of flunixin may not represent pre-emptive analgesia because of the prior abomasal displacement. Another study was designed to investigate the effect of preoperative and 24-hour postoperative administration of flunixin meglumine (1.25 g IV) on postoperative recovery of cows having surgical correction of left displaced abomasum.⁸ In that study, cows receiving flunixin meglumine immediately before and 24-hours after surgery had significantly better appetite, defecation, and milk production compared with cows not having been given flunixin. Recently, meloxicam has been used for pain management in cattle for a variety of conditions. This NSAID has been shown to be effective in mitigating pain of castration and dehorning and the pharmacokinetics suggest that meloxicam should be effective for perioperative pain management as well.³⁰ Clinical experience has been positive with this drug when (0.5 to 1 mg/kg body weight) given orally, every 24 to 48 hours. Future research is needed to more fully elucidate the clinical indication for the use of meloxicam and other emerging analgesic drugs. Based on AMDUCA guidelines, the authors only use meloxicam when pain therapy, and not anti-inflammatory therapy, is needed. In cases of severe, prolonged pain when a pathologic pain state has become established, gabapentin can be administered as a complimentary drug to meloxicam as a multimodal therapy.⁹ The use of gabapentin (10 mg/kg, PO, q12hr) has been useful in cases of deep digital sepsis and septic arthritis as a tool to dampen the exaggerated central nervous response to the limb pain. Multimodal therapy may also utilize opioids (Table 2) and various epidural analgesic strategies (Table 3) to achieve the desired effect.

Advances in Abomasal Surgery

Veterinarians treating dairy cattle commonly diagnose displacement of the abomasum. However, displacement of the abomasum (DA) is a relatively recent phenomenon with respect to the development of the veterinary profession. The earliest reported cases of DA were by Carougeau and Prestat in 1898 and Fincher in 1927, but this disease did not become commonly reported until after the 1940's.¹⁰⁻¹⁶ Prior to that time, DA's either did not occur, were not diagnosed, or were not described as such. Begg reported three cases of left sided displacement of the abomasum (LDA) of which one cow died of peritonitis following surgical reduction of the abomasum and two cows returned to normal after withholding all food for 48 hours.¹² Jones described manual correction of LDA without stabilization of the abomasum in two cows.¹³

The incidence of diagnosis of displacement of the abomasum increased greatly after the mid-1960's and is now an internationally recognized problem of dairy cows. Displacement of the abomasum is commonly referred to as a "disease of high milk production". This statement may be justified in that as dairy cows have been selected for genetic improvement based primarily on milk production, these same cows may have been selected into a high-risk group for development of DA. In 1971, Wallace reported that LDA occurs most commonly in 4 to 6 year old Holstein cows during the first 6 weeks postpartum.¹⁷ Historical factors found to be common among cows with LDA included hypocalcemia (12.4%), ketosis (42%), metritis (41.2%), retained fetal membranes (30.1%), and various stressors (11.7%). Also, LDA appeared to be more common among cows having twins. Physical examination findings common among cows with LDA included metritis (43.5%), mastitis (19%), enteritis (7.3%), and retained fetal membranes (4.8%). Research documented since this study generally has concurred with these findings.

Recently, "two-step" laparoscopic abomasopexy has been described^{18,19}. The "two-step" laparoscopic abomasopexy technique offers advantages of both laparoscopy and blind roll and tack techniques, without the disadvantages associated with the blind roll and tack technique alone. It combines the minimal invasiveness and visual control for abomasal positioning and fixation offered by laparoscopy, and the speed and minimal invasiveness of the roll and tack techniques¹⁸⁻²⁰. The "two-step" technique first involves the laparoscopic guided toggle bar placement within the abomasal lumen through the left paralumbar area in the standing cow, followed by laparoscopic suture retrieval through the right paramedian area while the cow is in dorsal recumbency¹⁸⁻²⁰. Two different one-step laparoscopic abomasopexy techniques have been reported and purported to simplify the use of laparoscopy to accomplish abomasal fixation.²¹ This technique is done similarly to Step 2 of the "two-step" approach except that the cow is placed in dorsal recumbency, and the right paramedian area is aseptically prepared.

The outcomes of the "two-step" laparoscopic abomasopexy compared to traditional right paralumbar omentopexy have been described.²² Ninety-six cows, divided into equal groups, had surgery for LDA. The surgical time for the laparoscopic abomasopexy is 10.2 minutes shorter compared to the traditional laparotomy. Post-operatively, cows that had a laparoscopic abomasopexy had significantly higher feed intakes (roughage +0.61 kg, concentrates +1.24 kg), and their milk yield rose significantly higher (+1.82 kg) compared to the traditional laparotomy group. These investigators suggested that less postoperative pain is experienced by cows that had the laparoscopic abomasopexy; therefore these cows had better feed intake and consequently improved milk yield. Furthermore, this procedure is performed without routine antibiotics, unless concurrent disease (ie metritis) indicated otherwise. This significantly reduced treatment costs and reduced the risk of antibiotic residues compared to the cows that had a traditional laparotomy for correction of LDA. The complications noted for laparoscopic abomasopexy include: premature suture breakage (2 cows), peritonitis (2 cows), and myositis at the abomasopexy site (1 cow).²² In the same study, 1 cow developed an incisional infection where a right paralumbar omentopexy had been performed to correct a LDA. These investigators did not indicate where the suture broke. It is possible either tying the suture around the stent in such a fashion that if one suture broke the other would still hold the toggle and stent in place, or placing a second toggle suture bar at the time of surgery would eliminate this complication. Peritonitis has been recorded as a surgical complication of laparoscopic abomasopexy^{20, 22}. However, peritonitis can also develop secondary to non-surgical complications, such as abomasal ulcers, traumatic-reticuloperitonitis, and metritis. Unfortunately, concurrent diseases were not recorded in either study.

One report from the University of Utrecht in the Netherlands suggested that the "two-step" laparoscopic abomasopexy technique replaced the right paralumbar omentopexy as the standard approach for correcting LDA in 1998.²⁰ These investigators reported on the results of 108

laparoscopic abomasopexies performed under field conditions. The data from 84 returned owner questionnaires are included in this study. The survival rate is 76%, with 57 cows starting a new lactation; 40 cows are not bred, and the fates of 11 cows are unknown. Unfortunately, these results are not compared to their previous results using the right paralumbar omentopexy technique. Peri-operative conditions such as ketosis, fatty liver, retained fetal membranes, metritis, and mastitis remain a challenge to manage post-operatively, and can adversely affect LDA outcomes, regardless of the LDA corrective technique performed. The long term anatomical assessment of the laparoscopic abomasopexy correction for LDA has been reported.²³ Nine weeks post-operatively (6 weeks after the toggle suture had been released), the fixation site between the abomasum and the ventral body wall had stretched up to 15 cm. Therefore, these cows may be at higher risk of LDA recurrence. These investigators suggest that a second toggle suture bar and maintaining the suture longer may produce more permanent adhesions, and consequently less likely for LDA recurrence. Recently, laparoscopic suturing of the abomasums was described to provide a broader, more secure fixation of the abomasum.^{24,25} Although more technically demanding than laparoscopic toggle-pin fixation, this technique may provide for longer term fixation in dairy cows. An advantage of the "one-step" technique over the "two-step" technique is the ability to perform the laparoscopic abomasopexy in the event of a misdiagnosis. This then raises the possibility of performing prophylactic laparoscopic abomasopexies in breeding age heifers. Prophylactic abomasopexies on farms that have high rates of LDA might reduce any adverse events or complications associated with LDA correction in the early post-partum cow.

Many advances in bovine surgery have occurred over the past 25 years including thelscopy, thoracoscopy, epiduroscopy, digital surgery, and cruciate ligament replacement. Although detailed discussion of these is beyond the scope of this proceedings, their contribution to the advancement of bovine surgery should not be discounted. Only through the dedicated efforts of individuals who passionately advocate for the advancement of clinical expertise can we progress. These individuals are some of the finest clinicians and scientists with whom I have ever been affiliated. I am grateful to be able to discuss these issues in highly respected forums such as the World Buiatrics Congress!

Table 1. Analgesic Drugs used in cattle.

Drug	Route	Frequency
Na-Salicylic acid (Aspirin)	PO	Q12-24h
Flunixin meglumine	IV	Q12-24h
Ketoprofen	IV	Q24-48h
Phenylbutazone	PO PO	Q24-48h Q48-72h
Meloxicam	PO	Q24 to 72h
Morphine	IV epidural	Q12h Q24h
Meperidine	SC or IM	
Buprenorphine	IM	Q12h
Butorphenol	SC	Q6h

Table 2. Epidural anesthesia for paralumbar laparotomy.

Drug	Onset of analgesia	Duration of analgesia
Lidocaine 2%	5 minutes	10 to 115 minutes
Xylazine	20 to 40 minutes	2 to 3 hours
Clonidine	2 ug dose: 19 minutes 3 ug dose: 9 minutes	2 ug dose: 192 minutes 3 ug dose: 311 minutes peak effect during 60 to 180 minutes
Ketamine 5%	5ml: 6.5 minutes 10 ml: 5 minutes 20 ml: 5 minutes	5 ml: 17 minutes 10 ml: 34 minutes 20 ml: 62 minutes
Procaine HCl 5%	8 to 20 minutes	45 to 127 minutes mean, 83 minutes

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Dairy Herd Health : a look at the veterinarian's changing role and the future of One Health

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This presentation will focus on preventive veterinary medicine in the area of dairy herd health. I will first examine how this field has evolved over time using a model proposed by the late Dr. Calvin Schwabe. I will then examine the current role of the bovine practitioner in ensuring herd health, especially in the collection of data for use in decision making. Finally, I will look at what the future holds for the veterinarian in dairy practice.

In general, the practice of veterinary medicine has evolved at different rates in different parts of the world. The tools used by veterinarians and the solutions proposed for medical issues also vary depending on the species and type of animal production. A variety of factors – historic, geopolitical, economic and social – come together to determine the role of the veterinarian in any given country.

The history and evolution of veterinary medicine have been the subject of numerous articles and books, many of which have focused on its relationship to human medicine and civilization. One thinker and researcher who has contributed greatly to our understanding of the evolution of preventive veterinary medicine is Calvin Schwabe. Dr. Schwabe was a veterinarian and professor at the University of California, where he founded the Department of Epidemiology and Preventive Medicine, now the Department of Medicine and Epidemiology. He passed away in 2006.

The epidemiological revolution in veterinary medicine

Our profession, like any other, is influenced by advancements in science and technology, along with changing perceptions of ourselves and our view of the world. Schwabe published two pivotal papers on the subject: The current epidemiological revolution in veterinary medicine. Part 1 and The current epidemiological revolution in veterinary medicine. Part 2. Both appeared in the journal Preventive Veterinary Medicine, the first in 1982 and the second in 1993.

In these articles, Schwabe presents a model for understanding the evolution of preventive veterinary medicine. He used the concept of “disciplinary matrix” to represent the research-practice complex underlying disease management in preventive veterinary medicine. Inspired by the ideas of Thomas Kuhn, he defined disciplinary matrix as:

“the complex of self-perceptions, assumptions, underlying theories, long and short-term goals, social and human motives, values and other beliefs, together with the infrastructures, strategies, tactics, techniques and other practices which members of any science currently share.” - Schwabe, 1982

Schwabe identified five “normal” phases in the evolution of veterinary medicine based on knowledge of disease causality. Today's veterinarians will be able to most easily identify with the two most recent phases, IV and V.

(IV) Campaigns or mass actions (1884-1960)

In this phase, the prevailing theory of disease causality is identified as “specific etiological agents” or infectious agents.

The crises that lead to phase V were: recognition of “problem herds” in

campaigns, the demand for economic justification, insidious and “production type” diseases, and the special needs of intensive production units.

(V) Surveillance and selective actions (1960-)

In this phase, the prevailing theory of disease causality is identified as “multiple interacting determinants (agents, hosts, environment, management)”.

Many of us are still familiar with phase IV and the fight against infectious agents. The medical sciences witnessed the introduction of antibiotics during this phase. This discovery had a lasting impact on both human and veterinary medicine, and on associated research in these fields. This phase saw the rise of paradigms centred around, among other things, laboratory diagnosis, vector control, mass communication, mass treatment and education. The passage to a new phase happens when anomalies and crises arise.

A particular phase of normal veterinary science waned when its researchers and practitioners began to encounter anomalies with respect to their currently accepted disciplinary matrix. - Schwabe, 1982

This leads to the formation of new and more adapted paradigms that eventually supplant the old ones. This passage occurs naturally but gradually; while some of us are ready and prepared for the change, others face greater challenges, depending on the expertise available and where they practice. Schwabe noted that changes in activities and perceptions are typically slow to be recognized and that it takes time before they are accepted by a critical mass of researchers and practitioners.

“As one normal veterinary science phase [supplants] another, those aspects of formerly dominant strategies, goals and infrastructures deemed to still possess value [are] retained, but subordinated to those of the new phase..., thus expanding the numbers of tactical permutations and combinations in the veterinarian's totally available armamentarium.” - Schwabe, 1982

This predicted evolution/revolution in paradigm appears at different rates and to varying degrees in different parts of the world. Likewise the role played by veterinarians in fulfilling national or regional needs varies depending on wealth, available technology, public awareness, education level of the population, and food production systems. But whatever form the change takes, it is clear that we always build upon previously acquired knowledge and that the successes of the previous phase are retained. While the emphasis shifts toward new paradigm solutions, the previous phase still exerts its influence. Note that it is veterinary practitioners that are responsible for maintaining cohesion through paradigm shifts, such that the profession can continue to meet its objectives and fulfill its role.

“I predict further, that improved productivity of animals will be as active an area of veterinary medicine-services delivery then, as are animal survival and health maintenance today” - Schwabe, 1993

The observations made by Calvin Schwabe in 1982 were based on changes that were already happening in veterinary practice and research. These changes were facilitated by developments in communication technologies and computer science. The introduction of computers has changed not only many aspects of our daily lives, but also the scientific approach in medicine. Statistics, modeling and information access have all been reshaped by the new technologies. Veterinary medicine is deeply involved in the epidemiological revolution, with risk assessment, evidence-based medicine and now modeling being applied to diagnosis and prevention. Change is happening at different levels of intervention, from the animal right up to the human population.

In 1993, Schwabe was critical of advances made at “herd-level practice,” which he considered as “an ongoing form of field research (surveillance, with appropriate analyses of data) in relation with production”. He blamed “insufficient communication between veterinarians trained in epidemiological diagnosis and those who are not”. This problem still exists, although there has been considerable progress.

Decision making – EVIDENCE BASED MEDICINE – BIG DATA

Faced with multiple risk factors for disease in a herd or population, decision makers must decide which factors to prioritize and how to minimize their effects. When prioritising, many factors have to be considered: herd productivity (economic), animal welfare, product quality, environmental impact and consumer opinion. Decision makers also need data to support their decision making. With the aim of improving dairy herd performance, Canadian researchers and practitioners developed a database to store the health information of individual cows (Bouchard et al., 1991). These data are used to generate information at both the herd and regional level. Data collected in such large databases can also be used to generate information through retrospective studies.

It is important to distinguish between data, information and knowledge. Stand-alone data like “25 kg of milk” does not provide any information unless it is related to stage of lactation or some other value or state. Data and information relate to a specific situation, “whereas knowledge comprises general statements about the world that are useful for explaining, predicting, or guiding future action... and is produced by the application of analytic methods on data and information.” (Ida, 2016). Data analysis using epidemiological methods will give the information and knowledge needed to make decisions and practice evidence-based medicine. “In contrast, big data approaches rarely involve protocol-directed data collection but aim to maximize precision and external validity by the dictum of ‘more data are better than better data’.” (Ida, 2016). Both evidence-based medicine and big data contribute to enlarging and strengthening the knowledge base of clinical medicine.

Models derived from retrospective studies conducted on large populations are already used to predict the risk of an event for an individual or population. In human medicine, modeling is used to predict the risk of heart attack for a given patient based on information about known risk factors. In veterinary medicine, Bouchard (2003) used such an approach to evaluate expected first-service conception risk based on the prevalence of previously identified risk factors combined with their measured effects in dairy herds. Risk factors studied included lactation rank, dystocia, retained placenta, metritis, ovarian cyst, mastitis, lameness, production levels (3 groups), and calving to AI interval (4 groups).

Another example is Bates and Dohoo (2016), who used individual cow records to evaluate risk factors for mastitis. Their retrospective longitudinal cohort study included 18,162 cows from 30 commercial dairy farms on South Island, New Zealand. Risk factors studied were: age, breed, length of dry period, farm, herd size, yield and individual somatic cell count status 30 to 60 days before the end of the previous lactation, rainfall at calving, and number of calvings on the same day. The results of this study could be used to predict expected mastitis level in similar herds.

We need to stress how important it is for veterinarians and producers to collect and eventually combine health record data to obtain valuable information for use on the farm. The knowledge acquired from such data can be used in decision making aimed at eliminating or reducing risk factors through targeted interventions in herd management. Collaboration with researchers is needed for data analyses related to the detection of causality, risk assessment, classification, prediction, modeling and simulation.

LEVELS OF INTERVENTION FOR THE VETERINARY PRACTITIONER

Decisions concerning animal health have to be made at different levels. Veterinarians participate in such decisions for the benefit of the animal, the producer, the transformation industry and, above all, the human population.

The first level of intervention involves the animal and producer. Since the beginning of veterinary practice, practitioners have been confronted with issues at this level. In the beginning, the veterinary practitioner is called

upon to prevent death and obtain an acceptable level of production by re-establishing good health status. The veterinarian's skills and knowledge in this area have improved over the centuries. In most countries, we now expect veterinarians to keep a written report of their interventions on an animal, including identification, clinical signs, diagnosis, treatment and so on.

The individual health record is considered the basic tool for practicing individual medicine. This record should include all pertinent data on a single animal, rather than simply a list of interventions (diagnosis and treatments) done on the herd. It includes data on demographics (birth, calving, insemination dates), preventive actions, diseases, treatment and production for the animal. By having complete data for each individual animal, the practitioner can evaluate the entire herd.

The second level of intervention involves the herd. Analysing health records provides information on the performance of the herd and the presence of disease in the herd. This is possible thanks to epidemiological and statistical methods. Researchers can develop algorithms to evaluate health status and progression of disease in the herd. Computers and software are the tools of choice for performing such analyses. The veterinary practitioner also has a significant role to play in this area, often being responsible for data entry at the farm level in order to ensure it is complete and complies with quality control programs.

The third level of intervention involves industry and public health authorities. Individual animal and herd information can ultimately be used at the regional and national levels. Data reports on an annual or continuous basis yield valuable information for the industry, while follow-up studies provide information on the impact of interventions. Many countries have drawn up standards and implemented traceability systems. In general, the availability of standardized and validated data is essential for decision makers to intervene at this level.

Note that research can be applied at all of these levels. The existence of research networks, collaborative relationships and access to common databases are all essential in such research.

FUTURE – ONE HEALTH, ARE WE READY?

With increasing consumer awareness and demands, we are seeing an increase in regulation by governments, public organizations and industry. As a result, veterinary expertise, research and knowledge is increasingly being solicited. It is clear that responsibilities and duties must be shared within the veterinary profession and with other professionals. The traditional role of the veterinarian has expanded from simply attending to sick animals to encompass whole new areas of expertise. This specialization has been underway for some time now, such that today the American Veterinary Medical Association (AVMA) recognizes 22 veterinary speciality organizations comprising 41 distinct specialties. Note that not all of these specialty domains apply to farm animals. Most of the specialties in laboratory diagnosis are solicited in food animal production. Still, more communication between the bovine practitioners and the laboratory specialists is desirable for a better integration of the available laboratory diagnostic tests and the development of new ones.

In disease control and health management, decisions have to be made at different levels of organization. At each level, one or more decision makers are involved. Their goals will vary according to their responsibility and objectives, but clearly all decision makers have to be able to communicate effectively in order to be efficient in their interventions.

The concept of “One Health” was introduced almost a decade ago. (As mentioned earlier, “changes in activities and perceptions are typically slow to be recognized”.) As more and more veterinarians work with other professions and scientific realities, they share knowledge and concerns. We now see more veterinarians in the public administration of not only animal, but also human, health. In my country, Canada, we have witnessed the establishment of a new university school of public health in which veterinarians are actively involved (ESPUM, University of

Montreal). Also, the latest veterinary faculty established in Calgary, Alberta is associated with a human medicine school. It integrated comparative health and biomedical research to strengthen the connections between human and animal medicine following the concepts of Virchow in the late 19th century and Schwabe by the end of the 20th century and committed to apply the One Health concept. We also see veterinarians in the Public Health Agency of Canada. This continued and increasing presence of veterinarians in research and public health administration will lead to the creation of better links with veterinary practitioners and the more efficient management of public health.

The collaborative effort of multiple disciplines working locally, nationally, and globally to attain optimal health for people, animals and our environment. - American Veterinary Medical Association, 2008

Networking and animal health databases will gradually come to play an increasing role in the management of public health. In a world of increasing population density and rapid easy communication, veterinarians have to react quickly to get an accurate diagnosis and implement targeted actions. These actions will often need to take place at the farm level, as for example, in the case of pathogen control and the use of antibiotics.

Big data are a distinct “cultural, technological, and scholarly phenomenon” (Boyd) centered on the application of machine learning algorithms to diverse, large-scale data. - Ida, 2016

Basic and applied research have contributed greatly to making diagnostic tools increasingly precise and adapted to field use. But it is important that we veterinarians also contribute to the development and application of new diagnostic tools like laboratory diagnostic tests. We need to establish stronger communication links between research centres, diagnostic laboratories and the field. The task of creating more efficient disease, production and public health systems is a responsibility to be shared between producers, a variety of professionals, decision-makers and the public. It is a state of mind that requires continuous communication, effort and education.

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New Zealand Dairy Herd Reproduction: Our role in a new pandemic

Tom Brownlie

Introduction

In the last two decades, our dialogue on dairy herd reproduction has changed as our access to objective herd information has increased. We now largely accept there has been a decline in reproductive performance internationally (Lucy, 2001). Early reports of falling conception rates were published in 1998 in Ireland (O'Farrell and Crilly, 1998) and the US (Butler, 1998) and 2000 in the UK (Royal et al., 2000). It has been speculated that selection for both production and the increased use of Holstein Friesian genetics in herds are primary reasons for this decline (Berry et al., 2013; Evans et al., 2006; Olori et al., 2002) although some historic data is limited by its incompleteness.

Evidence of a decline was first reported in New Zealand in 2006 (Harris, 2006) though there was little evidence of increased use of Holstein genetics (approximately 37% of the national herd) and only a weak link with high milk production when compared to other countries (Grosshans et al., 1997). New Zealand (alongside a growing number of Irish herds) operates a seasonal calving system in order to align peak milk production with peak pasture cover (Verkerk, 2003). New Zealand cows must calve and reconceive during a limited breeding period in order to be retained in the herd. As a result, NZ has had the shortest average calving interval amongst the ICAR countries (<http://www.icar.org>). The proportion of animals pregnant to the first 42 days of the breeding period (the 6 week in-calf rate) is considered the industry standard reproductive outcome measure in New Zealand (Morton, 2000a; Morton, 2010). However, to be measured accurately; all eligible animals in the herd should have a timely pregnancy diagnosis from which the conception date of pregnant animals can be accurately estimated. Early evidence of reproductive decline in New Zealand was made in the absence of accurate pregnancy data (Harris, 2006). Consequently, the decline was identified in the proportion of animals calving in the first 42 days of the two successive breeding seasons (Figure 1). This metric does not account for animals that do not have a subsequent calving in the herd; nonetheless, a marked decline is still evident.

This decline has been the justification for extensive research and focus from industry stakeholders: *Twenty years on, let us reflect on where we have got to?*

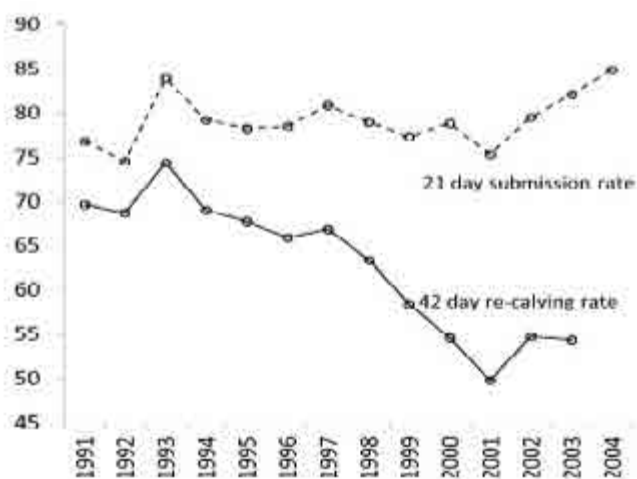


Figure 1. Mean 21 day submission rate (proportion of herd bred in the first 21 days of breeding) and 42 day re-calving rate (proportion of herd calving in the first 42 days of calving periods) of a sample of 221,224 cows from the LIC Sire Proving Scheme database between 1990 to 2004 (Harris 2006)

Advances over the last few years

Three key changes in the dairy industry have had a direct or indirect impact on dairy herd reproductive performance in New Zealand:

- The launch of a national extension programme (InCalf) for dairy farmers,
- the addition of a fertility trait in the National Breeding Value Index
- the accelerating use of technology on farm creating broader and deeper data streams.

i. A National Extension Programme: InCalf

InCalf is an extension package of resources, tools and training for both dairy farmers and their advisors. It was originally developed in Australia (Britton et al., 2003) and adapted for the New Zealand system in 2007 (Blackwell, 2008). InCalf offers advisors a structured framework to be used at critical times throughout the seasonal dairy year and focuses on eight key risk factors considered important for reproductive performance (Morton, 2000b). Its release was widely promoted in the national agricultural press and through stakeholder roadshows and the advisor training has been well attended. There are currently 550 trained InCalf advisors (www.dairynz.co.nz), of which approximately 75% are veterinarians. If we accept that only predominantly large animal practitioners have undertaken the training then over two thirds (68%) of all New Zealand large animal practitioners are also InCalf advisors (Anonymous, 2013b).

Following its release, the effect of participation in the InCalf programme was quantified using a large scale multi-centre multi-year randomised controlled study (Brownlie et al., 2015a). The study evaluated InCalf primarily on change in the 6 week in-calf rate, a key performance indicator of InCalf. Participation in the programme was associated with a modest but sustained 2% improvement in 6 week in-calf rate over herds that did not participate. In order to better understand the process behind this effect, the study quantified the effect of participation on key risk factors (Brownlie et al., 2015b) and conducted extensive interviews to determine the attitudes, values and goals of the participants (Brownlie et al., 2011). This paper will consider each of these steps from a New Zealand perspective.



Figure 2: Simplified schematic of the proposed pathway to achieving change in an end-result through modifying or removing risk factors (in a farming context this is commonly adopting new or modified existing management practices) as a result of changes in Knowledge, Attitudes, Skills and Aspirations (KASA). The process is adapted from behavioural theory and comprises the top three tiers of Bennetts Hierarchy of Evaluating Agricultural Extension (Bennett, 1975)

ii. Genetics: Fertility BV

Alongside the national extension strategy, a Fertility Breeding Value was incorporated into the New Zealand Breeding Value in 2002 (Harris, 2006). The Breeding Value is a genetic score based on nationally available data that compares certain traits within contemporary daughter groups and years, to help support breeding decisions. The Fertility Breeding Value is based on reproductive traits including being bred in the first 21 days of the breeding period, calving in the first 42 days of the calving period, body condition score in the first 60 days of lactation and milk volume. The traits are identical to those used to assess herd performance and hence it is inappropriate to incorporate Fertility Breeding Value in models of management practice. However, breeding selection should be acknowledged as a hidden variable inherent in models of the effect of management practices over time. The Fertility Breeding Value has low heritability (estimated at 9% heritable trait when associated with its predictor variables; Harris pers comm); but the effect is cumulative over time (Bowley et al., 2015).

iii. Technology:

The contribution of technology cannot be ignored when considering change in the dairy sector. Its adoption is leading to a pandemic of data largely driven by automated collection of behavioural, biological and environmental data. Data capture is intrinsic in most new technology yet on its own, can quickly become obsolete if not integrated with other streams of data. In technology terms, longevity requires integration. Commendably, some veterinary businesses are already growing off the back of aligning these data (Infovet (Zoetis Inc.) and VetImpress (Farmvet Systems Ltd.).

Figure 3 illustrates the increase in pregnancy testing events that are now captured in the LIC proprietary database in New Zealand. These data are recorded on farm through multiple mobile sources some of which are competing companies. The pressure to integrate in this case arises from the requirement that data be available on the predominant on-farm software platform in New Zealand in order to have perceived value.

Integration often occurs before any value is added to the data through expert interpretation or analysis. The challenge for rural professionals is how and where to add this value.

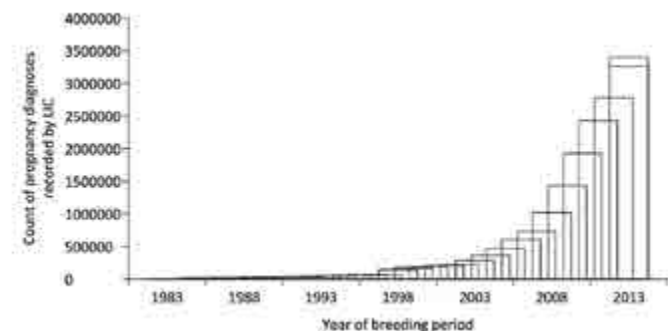


Figure 3: Annual increase in national pregnancy diagnosis recording evident in the LIC proprietary database (LIC, New Zealand).

1. End Result: improving 6 week in-calf rate

Important key performance indicators for reproductive performance in seasonal systems are commonly proportions (e.g. the incorrectly termed 6 week in-calf rate). The challenge when calculating these is ensuring that both the numerators and denominators are precise and comparable.

By applying consistent rules to the New Zealand national dataset, a unique picture of the reproductive performance for all herds in can be described. For this purpose, 39,379,095 breeding's, 29,337,967 calving's and 18,128,116 pregnancy diagnoses were used to describe reproductive performance in 12,713 distinct herds from 2009 onwards. Animals that ceased contributing any events yet had no removal record were right censored in their last year of contributing (comprising approximately 12% of the national dataset). The results suggest that from 2010 onwards there has been a modest, incremental improvement in the overall reproductive performance of New Zealand dairy herds (Figure 4 a-c).

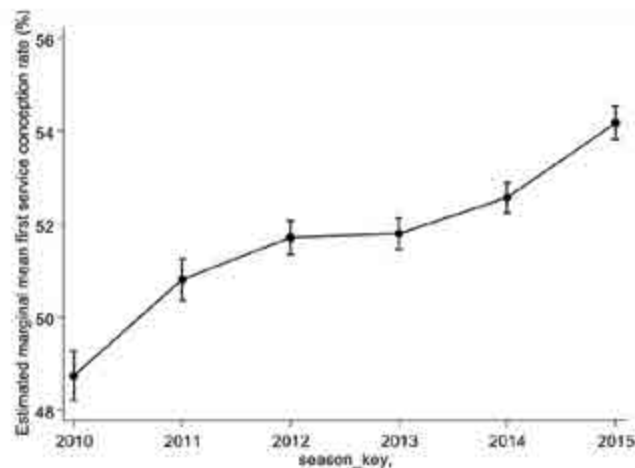
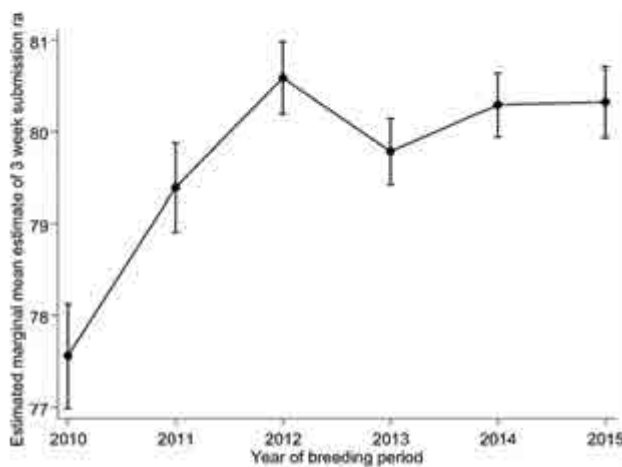
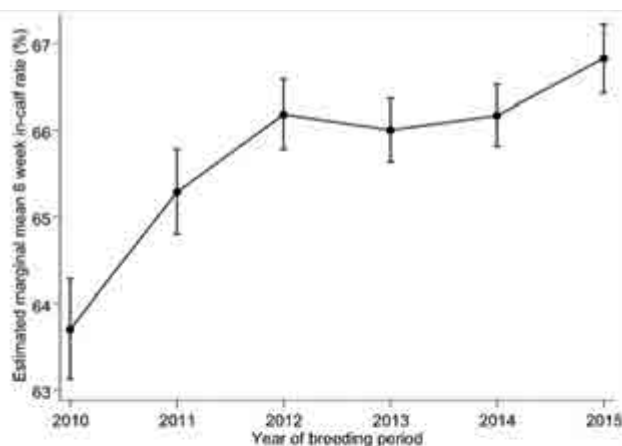


Figure 4. a –c. Estimated marginal mean outcomes from three herd-year level linear regression models of 6 week in-calf rate, 3 week submission rates and conception rates for New Zealand dairy herds eligible to generate cumulative pregnancy life tables (i.e. ≥80% of herd having an aged pregnancy test or confirmed not pregnant under 170 days after the start of breeding)

The economic benefits of a high 6 week in-calf rate are relatively simple to estimate and make the in-calf rate an appropriate target for overall herd performance. However, to benchmark herds on this measure alone is insensitive to the levels at which risk factors affect reproductive performance (Xu, 2003). Variation in performance occurs at region, herd and cow level in different amounts. To improve performance, effort should be concentrated where greatest variation is found.

To illustrate this, data from the New Zealand National Herd Fertility Study were used to create multilevel mixed-effects logistic regression models to determine (a) 6 week in-calf rate, (b) 3 week submission rate and (c)

	Intraclass correlation coefficient (95% CI) as a percentage		
	6 week in-calf rate	3 week submission rate	First service conception rate
region	<0.01(<0.01 - <0.01)	0.07 (<0.01 - 33.66)	0.02 (<0.01 - 12.04)
herd within region	3.30 (2.51 - 4.21)	7.50 (5.87 - 9.53)	2.10 (1.60 - 2.76)
cow ^a	96.75	92.50	97.88

Table 1. Intraclass correlation coefficients presented as percentages from three multilevel mixed effect logistic regression models of three measures of reproductive performance where herd and region level were fitted as random effects from data on cow-years enrolled in the New Zealand National Herd Fertility Study during the 2009/10 and 2010/11 years. Repeated measures between years was removed by including the year as a fixed effect in the models

^a residual variance found at cow level was derived by subtracting coefficients from region and herd level from one

first service conception rate, the latter two being considered the drivers of in-calf rates. No further covariates were included in these models. Intraclass correlation coefficients were derived from these models (Table 1). In mixed effect models, intra-class correlation coefficients measure absolute agreement between outcomes at nested levels within the model. Conditional on the year, reproductive performance measures barely correlate within the same region, and only very slightly within the same herd and region. The remaining residual variance therefore resides at cow level. Thus to improve herd or regional reproductive performance, identifying risk factors for individual cows will be the most effective route. Advisors should be aware of cow-level risk factors and which ones can be measured and improved.

2. Risk factors

Cow-year-level risk factors affect the reproductive performance of a cow within a lactation (such as periparturient mastitis or calving date relative to the start of the breeding season) but can also affect herd-level reproductive performance, if a substantial proportion of the herd is affected (Evans and Walsh, 2011; Xu, 2003). However, the relationship between cow-year-level risk factors and reproductive performance cannot simply be inferred at a herd-year-level without the risk of atomistic fallacy (for example, the effect of Jersey breed as a cow is not necessarily transferable to a herd level as it is easily confounded by the age structure of the herd). Furthermore, some cow-year-level risk factors are a consequence of the same management factors at a herd-level, for example, proportion of herd meeting pre-mating body condition score targets and herd mean milk protein at first herd test are both reflective of herd nutrition and to a lesser extent, genetic status. Many of these factors are also confounded by cow-level factors (e.g. cow breed) and certain cow-year-level risk factors (e.g. cow age and calving date relative to the start of the breeding period). So some factors are associated with reproductive performance measures directly as well as interacting with other risk factors (e.g. association between different cow breeds on milk production parameters).

In New Zealand, the National Herd Fertility Study provided accurate data on a wide range of risk factors from 134,880 cow years from 2009 to 2011 in four dairy regions of New Zealand (Brownlie et al., 2014). Table 2 presents the outcomes of the same multilevel mixed effects models presented in Section 4, with addition of key risk factor following reverse stepwise modelling. Table 4 uses many of the same variables summarised at a herd-level. These results demonstrate that multiple factors including age, breed and date of calving relative to the start of the breeding program strongly affect reproductive measures at both levels, yet other factors do not transfer their effect to the herd-level, e.g. proportion of calving's given assistance or cows treated for mastitis during breeding. Interestingly, milk production parameters (milk protein percent and milk fat (kg)) explain greater variation in reproductive performance than body condition score alone.

So, given this wealth of information and the growing access to clear messages; *how has the New Zealand veterinary profession responded?*

3. Knowledge, Attitudes, Skills and Aspirations

If we follow the framework for behavioural change that we introduced in Figure 1, we should next examine the attitudes, priorities and perceived constraints of decision makers on these herds (Fishbein, 1975). However, let us instead look to rural professionals (i.e. veterinarians) who advise these decision makers. Veterinarians are consistently identified as the key advisors on herd reproduction and health (Burke et al., 2008) and for over 20 years the veterinary profession has been encouraged to prioritise formal advisory work (Mee, 2010). Today, as agriculture moves across the technological epoch, the imperative to adapt will be due to new pressures: Smart algorithms are rapidly evolving that will automate on-farm decision making and this will most likely erode the veterinary role as custodian of reproductive performance.

The growing importance and depth of information being collected on-farm means that we will invariably have to become consumers of these data in order to remain relevant.

We will end up paying for access to these data unless we can trade our expertise to become part of this process. Where it becomes likely that we will need to invest, we need to know how to make the investment work well for us. Unlike other challenges the profession has faced, this one presents many opportunities.

As part of an engagement process, sixteen purposively selected veterinarians from across New Zealand were inducted into a Design Thinking process where the future role of veterinarians in the Data Value Chain (Gustafson and Fink, 2013) was explored. Design Thinking is an established, structured design process that uses informal interviews to create concise statements of participants' perceived constraints and opportunities (Kimbrell, 2011). Participants were given access to data stored in the LIC proprietary database and asked how to generate value from these data.

Four key statements were described using this process:

1. Data that vets collect on farm needs to be integrated with farmer software in order to be valuable.

Currently, many veterinary practices across New Zealand invest a considerable sum in a software service principally to upload pregnancy diagnoses onto farmer software and thereby increasing the value of their diagnostic service. Study participants felt that this investment was justified if it simply maintained a lead in a highly competitive market.

2. Benchmarking key performance indicators is the starting point of most advisor conversations

Participants felt that a key role of reproductive data was to provide benchmarking. Benchmarking provided a motivational tool for farmers and vets; provided evidence of change and was used as a common starting point for advisory work. Local benchmarks or benchmarks from similar farming systems were considered the most engaging although not easily available.

3. Allow veterinarians to perform exploratory data analysis will create value

Exploratory data analyses are normally embarked upon in response to poor performance. It is a service offered by an advisor to identify specific gaps in herd performance to target professional intervention.

4. Generate a tangible chargeable product to justify the cost of data

Most participants did not have clear plan to generate sustainable income from the data in which they had invested. Most participants favoured customised reports for herd owners if they were not time consuming to prepare. However, if these were standardized and inflexible, their long term use would diminish. Participants felt that data access should increase their competitive advantage and that they were prepared to continue investment in employee upskilling once greater access to data was secured.

Confidence around the role of data in participants businesses was highest in the first two statements but diminished in the second two. This reinforces the perception that the veterinary industry does not have a clear business case yet for the use of the large amount of data being generated. Fortunately, veterinary advice remains a valuable commodity. Veterinary advice on reducing reproductive risk factors augments automated data collection and empowering veterinarians to integrating with these systems is beneficial to more than just veterinarians. It is now up to the veterinary profession to ensure that we are willing to participate and even guide this new and exciting technology.

Conclusions

The reproductive performance of New Zealand dairy herds has been a focus for many industry stakeholders for almost 20 years. The investment in science, extension and technology is likely to be reflected in the incremental improvement in performance. However, a fundamental shift is still required if New Zealand stands any chance of reaching its industry target of a national average 78% 6 week in-calf rate in the next 4 years (Anonymous, 2013a). This target seems unachievable unless we return to cow-level management as our best strategy. Armed with objective information on clients' herds, veterinarians have a key role as advisors.

The challenge for the profession is to become recognised for their position in this data value chain: We must engage with other people in the farmer network and become innovative and collaborative rather than hope that the status quo pervades. After all, we ought not to use data only as a *drunken man uses lamp posts – for support rather than for illumination**.

*(A. Lang)

For tables, acknowledgements, and references please go to the following webpage: <http://imgpublic.mci-group.com/ie/PCO/TomBrownlie.pdf>

Health Planning for the Beef Cow Herd

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Introduction

The planned approach to managing animal health through analysis of production data and risk assessment has supported intensive livestock production systems for decades (Blood, 1979). The veterinarians' skill and knowledge has given them a pivotal role in supporting efficient food production from our livestock whilst safeguarding their welfare. This relationship has been well-developed in those sectors that have driven to improve efficiency, but is poorly developed in the beef cow industry. Although production from the beef cow herd is an important area of agricultural output throughout the world, this production system is inefficient and long term profitability is rarely achieved without the help of direct payments (Deblitz 2012). There is a higher demand for maintenance per kilogram of output than any other livestock sector arising from the high maintenance costs of the parent herd (Morris and Smeaton 2009) and the long period from birth to slaughter. Relative profitability has been shown to be closely related to physical output, but estimates of the average number of calves weaned expressed as a percentage of cows mated varies around 87% in most years in Great Britain (QMS, EBLEX, and HCC) and an average of 82% has been quoted for the New Zealand herds. In contrast targets of 94% in Great Britain (MLC 1998) and 92% New Zealand (Morris and Smeaton 2009) have been cited.

Nevertheless the beef cow's ability to use poor quality herbage to produce and rear a calf ensures that beef cow production has a place in areas where there are limited alternatives for land use such as in the tropical rangelands of Queensland (Gleeson et al 2012) or the temperate rangelands of North America. Furthermore beef cow herds also complement more efficient production systems such as fat lamb production in New Zealand where the cows can be used to improve pasture management (Pleasants et al 1991) or in areas of arable production where the by-products, particularly from cereal crops, can be fed to cows.

A further area of concern stems from this biological inefficiency: livestock production is a significant contributor to greenhouse gas (GHG) emissions and ruminants have a particularly high impact (Gill et al 2010). In beef cow production systems that use poor quality forage the proportional output of methane is higher and while large within breed variation is seen in both feed conversion efficiency and GHG emission it remains difficult to mitigate this environmental impact to any degree without improving production efficiency (Nguyen et al 2013).

Improving the efficiency of production will not only increase the gross margins of producers, but will reduce the GHG emission per kg of meat produced from the beef cow herd (Harrison et al 2016).

Improving the efficiency of beef cow herds.

Output efficiency for beef cow herds is measured as kilogram of calf weaned per kilogram of cow mated and an ambitious target is 0.5 kg per kg per year. The elements of this are the fertility of the adult herd, the ability of the calf to survive birth and to maintain its viability thereafter and the ability of the calf to achieve growth rates in excess of one kg per day on a combined ration of milk and grass. The cow should be able to use forage efficiently and to store additional energy as fat to reduce feed requirements through winter or dry seasons and additionally to exhibit strong maternal traits. For the effective use of resource cows should be reared to produce their first calf at two years of age, in all but the most challenging situations and to continue to produce a calf each year for nine years when mated for a restricted period of two months.

It is important to consider if enough is understood about the limiting factors to ensure these can be managed to promote efficient production? Whilst suckling does add two weeks to the time to onset of pulsatile luteinising hormone release), postpartum anoestrus in beef cows in the European situation (Busch 1998) and in North America (Werth 1996) is not a barrier to achieving the reproductive targets. The impact of energy supply on the reproductive function of the cows and on the interaction with growth in the young cows has been well described (Short et al 1990). Managing the cows' ability to use the cyclical supply of forage to store nutrients in body reserves has been facilitated by body condition scoring systems (Thompson et al 1983), creating a tool that can be used to achieve target body condition score at calving and breeding thereby ensuring that energy deficiency does not impair the return to oestrus. The other element of good herd fertility is the bull and sound methodology exists for assessing reproductive capacity in the bull (McGowan 2007).

The cow should also rear her calf and produce sufficient milk to allow the calf to achieve the growth target. Dystocia and calf mortality is the second largest area of loss and enough is already known about the factors that contribute to this (Waldner 2014). Working to reduce the occurrence of dystocia and ensuring the cows are fed to reach the target condition score will have the valuable consequence that sufficient quality colostrum will be available and that most of the calves will suck well and absorb sufficient immunoglobulin to mitigate the risk of neonatal disease. Infectious disease impacts on fertility and on calf survival and growth. Disease control is the final essential element required to promote beef cow production efficiency and enough is known to prevent disease incursion; to control contributory factors and to promote specific immunity.

These principles can be translated to simple messages. In Great Britain this has been done by creating a five point plan (QMS 2014) (table 1).

Health Planning

Health planning provides a framework for the delivery of veterinary expertise to the animal production unit to make best use of the resource for the benefit of the animals and the producers. Health planning should be the ideal way to improve production efficiency in the beef herd. In Great Britain cattle health plans were a formal document (Pocock 2004) that detailed the diseases that a herd were exposed to and the treatments and preventive actions that would mitigate the disease risks. The plan was reviewed each year. This was adopted by the farm assurance systems and a health plan became a mandatory requirement. The first problem with this was that the focus was on written detail on disease and not on analysis of production and disease occurrence and the consequent losses. The second drawback was that production of the document was seen as an end in itself and once produced the document was no longer referred to.

The challenge was to move from the static document on disease control to a dynamic process that embraced process management techniques to analyse production and identify areas for improvement. The collection of the requisite data to generate the key performance indicators is not a problem for most production systems, but the statutory requirements for the European beef cow herds is to record the number of animals on the farm, to register births within 27 days and to register deaths and movements off the farm as they occur. There is no encouragement to collect any other data. It is difficult to determine the key denominator, which is the number of females in the herd that were mated. Furthermore there may be no record of the calves that died before they were registered. To overcome these deficits we produced an approach to recording events in the herd to generate the key performance indicators, namely the percentage of calves weaned, the percentage of cows that calved, the percentage of calves born, the percentage of calves that died (at different stages of their life) and the percentage of cows that calved in the first three weeks of the breeding season and embedded this within a web-based database (SAC 2016).

The concept requires the beef cow farmer to enter data into the system; detail the current herd preventive actions and treatments and list any disease problem thought to be present on the farm. This can reduce the time required by the herd's veterinarian, as veterinary time need only start once the preparatory data has been entered. However, particularly given the seasonal nature of the production cycle the participating farmers enter the data so infrequently that they rarely establish familiarity with the software and find the process daunting. For many participating herds the veterinarian enters the data and thereby adds unnecessary cost to the process.

Hazard analysis critical control point (HACCP) methodology has been used in the delivery of veterinary advice to the dairy herd (da Silva et al 2006). This links risk management principles to operational management. and is appropriate because of the immediate public health aspects of milk production as compared to the sale of weaned beef calves, but by taking HACCP and aligning this with classic risk management methodology (OIE 2007) a methodology that is well-fitted to assessing productivity and managing risk within the beef cow herd can be constructed (table 2). This format allows the veterinarian to assess the risk of a particular hazard to the herd productivity, whether it is an infectious agent or a factor that may impact negatively on reproductive performance. Central to risk analysis is communication and prioritisation. In the case of health planning for the beef cow herd the identification of the main risks and discussion with the farmer allows effective prioritisation to achieve the greatest gain in a production system where financial resource is likely to be severely limited.

The focus of this discussion has been on improving output to increase the biological efficiency of the beef cow herd, but there are other important inefficiencies within these systems. While access to medicines varies from country to country there are many licensed products and feed additives that are used on farms without the veterinary surgeon being aware of their use. In the British situation there is considerable potential for the incorrect use of anthelmintics and mineral supplements. By requesting the farmer to detail this use the opportunity is created for the veterinarian to provide the advice to rationalise the use of these products such that the cost savings from this may exceed the professional fee for the health planning service.

Despite a powerful rational for health planning the gains have been slight and while herds in the top quartile show a level of performance that exceeds the target there remains relatively few herds participating in this approach. Cattle farmers in Britain have traditionally been slow to adopt health management strategies that have been shown to improve profitability. The improvement in milk quality did not result from a widespread acceptance of the simple messages on mastitis control, but followed payment penalties for total bacterial and somatic cell counts (Booth 2000). Indeed there may be a high level of scepticism or unwillingness on the part of the majority of farmers and veterinarians to be convinced that change is possible or that health planning is a tool that can be used to achieve improvement.

It may be that it is too difficult to record and engage with the process in the modern beef cow herds where the number of cows per herd has increased as the number of workers has declined. To overcome that barrier a different approach to recording events is required. Information and events should be recorded once and the information then is available for multiple applications, easing the workload rather than adding to it. There are statutory requirements for recording births deaths and movements and for recording medicines. Creating a hand held electronic solution to make use of herd animal inventory and medicine inventory whilst tying this to other events such as birth of dead calves and disease incidents and treatments is achievable. Mobile telephone technology now provides effective solutions for this data collection challenge.

Apart from the efficient use of data a further advantage lies in moving data analysis into real time allowing notification of events to the veterinarian. Clusters of deaths or events such as assisted calvings that may not have

triggered a call to the veterinary practice can be communicated through the system and allow early contact and discussion.

Conclusion: Produce from beef cow herds makes up an important part of the livestock output in many countries using land that has limited alternative uses as well as complementing more efficient production systems. The profitability of beef cow herds is low, stemming from low levels of biological efficiency, but also because the average level of productivity is low. There is sufficient understanding of the process and its limiting factors to manage herds to achieve outputs of at least 94 calves reared per 100 cows mated and yet the average output is consistently very much less than this. There is therefore a powerful rationale to improve the efficiency of production in order to both increase the returns from a poorly profitable enterprise and to reduce the greenhouse gas emission per kg of calf sold. As has been argued for decades, the veterinarian is well-placed to provide a service that will help achieve these goals through health planning and it is troubling that more has not been achieved. There is now a clear understanding that for health planning to be successful the process should embody data analysis and risk management and make the most efficient use of technology. It is envisaged that this will be achieved through the collection of data at the point at which events occur using hand held devices such as mobile phones with aggregation of data on a web-based database that facilitates health planning.

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Table 1: The five point plan for improving beef cow herd productivity

Heifer management Early breeding and target growth rates
Bulls: soundness and fertility Genetics and breeding soundness examinations
Manage cow condition and nutrition Target condition scores and avoid excessive weight loss
Avoid difficult calvings Select bulls on EBVs; prevent over fat cows
Maintain herd health Herd health planning and biosecurity

Table 2: Elements of HACCP and Risk Analysis

HACCP	Risk Analysis	Combined
Analyse hazards	Identify hazards	Identify hazards
Critical control points	Determine risk	Determine risk
Preventive measures	Prioritise risks	Prioritise risks
Monitor	Risk reduction measures	Critical Control Points
Action to take if monitoring indicates inadequacy	Monitor	Risk reduction measures
Verification system	Review	Monitor
Effective record keeping		Action to take if monitoring indicates inadequacy
		Review
		Effective record keeping

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Sustainable Agriculture

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Introduction

Agricultural sustainability (specifically livestock system sustainability) is one of the most highly-debated issues within food production. The sustainability concept may be partitioned into three components: environmental impact, economic viability, and social acceptability (United Nations, 2005). For a region, industry or system to achieve sustainability, all three components must balance - if one factor is misaligned, ignored, or the sole focal point, then long-term sustainability is impossible. Livestock provide high-quality food products in conjunction with a myriad of associated economic and social benefits to global communities. Nonetheless, the question is often raised as to whether milk and meat consumption is unsustainable. The majority of current research has focused upon the environmental component of sustainability, which given the positive correlation between resource use and economic viability, may also encompass farm profitability. This paper therefore concentrates primarily on the environmental aspect; with the understanding that reduced resource use also has positive economic and social impacts.

The Environmental Impact of Livestock Production

Environmental impact is a multifactorial issue. Although it is often discussed with sole reference to greenhouse gas (GHG) emissions, resource scarcity (specifically water, land, inorganic fertilisers and fossil fuels) may be argued to have a greater immediate effect upon food production. Nonetheless, the majority of studies to date have concentrated on GHG as the sole arbiter of environmental impact. Agricultural GHG emissions were estimated by Bellarby et al. (2008) to account for between 17% and 32% of all global anthropogenic emissions, with reports by the FAO (2006) and the World Watch Institute (Goodland & Anhang, 2009) concluding that animal agriculture contributes 18% or 51% of GHG emissions respectively. Global averages are somewhat meaningless, however, as they are not representative of any specific region or system and thus offer little opportunity for practical mitigation. For example, dairy production contributes ~2.7% of worldwide GHG emissions, with average emissions of 2.4 kg CO₂-eq/kg FPCM (fat and protein-corrected milk) at the farm-gate (FAO, 2010), yet significant regional variation exists, with values ranging from 7.5 kg CO₂-eq/kg FPCM in sub-Saharan Africa (average milk yield of 300 kg/y) to 1.3 CO₂-eq/kg FPCM in North America (average milk yield of 8,900 kg/y). It appears therefore that a negative correlation exists between milk yield and carbon footprint - as productivity increases, GHG emissions decrease per unit of milk.

The environmental mitigation effect of improving productivity is conferred by the "dilution of maintenance" concept (Capper, 2011a; Capper et al., 2008). Regardless of species, every animal within a herd or flock has a daily maintenance nutrient requirement plus an extra nutrient requirement for productivity (milk, meat or egg production; pregnancy and lactation). As productivity increases, the proportion of daily energy allocated to maintenance decreases and the maintenance requirement of the total animal population decreases. The nutrient requirement can be considered a proxy for resource use. As production system intensity increases, productivity improves, thus "intensive" systems tend to use fewer resources and have lower GHG emissions than "extensive" systems (Capper, 2011b). However, these improvements are not always well understood by consumers who often have a ideological view of the perceived advantages of historical small-scale agrarian systems compared to modern agriculture.

The dichotomy between popular perception and agricultural reality is exemplified by comparing the U.S. dairy industries in 1944 and 2007, as reported by Capper et al. (2009). A four-fold increase in milk yield per cow over this time allowed for a 59% increase in total milk production (53 billion kg in 1944 vs. 84 billion kg in 2007) whilst the national dairy herd was reduced from 25.6 million to 9.2 million cattle. This reduced feed use by 77%, land use by 90%, water use by 65% and conferred a 63% decrease in GHG emissions per kg of milk (Capper et al., 2009). Similarly, through improvements in genetics, nutrition and management between 1977 and 2007, the U.S. beef cattle industry increased average slaughter weight (607 kg in 2007 vs. 468 kg in 1977) and growth rate (1.18 kg/d in 2007 vs. 0.72 kg/d in 1977) which resulted in the total days required to raise a beef animal being reduced from 609 d (1977) to 485 d (2007). These gains resulted in considerable reductions in feed (19%), land (33%), water (12%) and GHG emissions (16%) per kg of beef over the thirty-year time period (Capper, 2011a). The U.S. pork industry has also reduced both resource use and GHG emissions per unit of pork over the past 50 years through similar mechanisms to those exhibited by the U.S. beef industry, i.e. increasing the number of hogs marketed from 87.6 million in 1959 to 112.6 million in 2009 (a 29% increase), from a breeding herd that has decreased in size by 39% over the same time period (Cady et al., 2013). Similar improvements have been demonstrated in many developed livestock industries, although their impacts are only now being quantified with reference to resource use.

Milk and meat from extensive forage-based production systems are attractive to the consumer wishing to buy food from "traditional" production systems, yet often use greater quantities of resources. Reduced growth rates and slaughter weights in combination with augmented methane emissions from a forage-based diet increase GHG emissions from 16.2 kg CO₂-eq/kg liveweight for feedlot-finished beef to 19.2 kg CO₂-eq/kg liveweight for pasture-finished beef according to Pelletier et al. (2010). If current intensive U.S. beef finishing systems were replaced by a wholly forage-based system, it would require an increase of 52 million ha of land to maintain current U.S. beef production and the increase in carbon emissions would be equal to adding 25.2 million cars to the road per year (Capper, 2012). Producing milk in organic production systems was found by Capper et al. (2008) to confer a 13% increase in GHG emissions per unit of milk (U.S.) and by Cederberg and Mattsson (2000) to increase GHG emissions by 15% (Sweden). By contrast, a comparison of organic and conventional poultry systems by Boggia et al. (2010) reported that organic poultry systems exhibited superior environmental performance.

Further Opportunities to Improve Sustainability

Management practices may be adopted to reduce specific environmental impacts, e.g. soil testing, installation of anaerobic digesters, or recycling water on-farm. However, a greater mitigation effect may be achieved through whole-system approaches that improve animal productivity and allow livestock to perform to their genetic potential. If a livestock system working at optimal efficiency would produce a set quantity of milk or meat in a given time, every loss of productivity within the system will reduce the milk/meat yield and increase both economic cost and environmental impact per unit of food produced. One major advantage of productivity-based approaches is that they are irrespective of system (e.g. intensive vs. extensive, conventional vs. organic), operation size or region, thus can be implemented globally according to resource availability and market considerations.

The aforementioned gains in milk, meat and egg productivity have had tangible results, however, in many livestock industries, animal bodyweight has increased concurrently with productivity gains, therefore daily resource use and GHG emissions per animal have increased (Capper, 2011a; Capper et al., 2009), although environmental impact per unit of food produced is reduced. If bodyweight in the supporting herd could be reduced without a loss of productivity, environmental and economic gains would result. Within dairy production, Capper and Cady (2012) reported that producing cheese from Jersey cattle (average

mature liveweight 454 kg) vs. Holstein cattle (average mature liveweight 680 kg) considerably reduced both resource use and GHG emissions. Within beef production, if mature beef cow bodyweight were reduced from 703 kg to 486 kg while maintaining the final carcass weight of the offspring, GHG emissions per unit of beef would decline by 13% (Capper, 2013b).

The introduction of genetic selection tools over the past century has allowed for greater rates of genetic improvement, however, these may have negative impacts, e.g. the conflict between increased milk yield in dairy cattle and declining fertility rates. Garnsworthy (2004) demonstrated that restoring fertility levels to those seen in UK dairy cattle c. 1995, or improving them to ideal levels, reduced methane emissions per unit of milk through a reduction in the number of heifer replacements required within the herd and thus a decrease in the herd maintenance requirement. Within the US, 89% of cows bear a live calf each year (USDA, 2009), and this number declines to between 50-60% in the extensive systems characteristic of Brazil, Argentina and Chile. Given that the cow-calf (suckler) operation contributes up to 80% of GHG emissions per unit of beef (Beauchemin et al., 2010) management practices and technologies that improve pregnancy rate offer significant opportunities. Capper (2013a) demonstrated that if calving rate increased from 60% to 100%, land use declined from 3.61 ha to 2.54 ha, water use from 682 x 103 litres to 508 x 103 litres, GHG emissions from 7,945 kg to 5,459 kg and feed costs from \$1,924 to \$1,412 per 363 kg beef carcass.

Is Vegetarianism the Answer?

Campaigns such as “Meatless” or “Meat Free” Mondays have been adopted by consumers who perceive that reducing meat consumption will improve their health or reduce their personal environmental impacts. Tobler et al. (2011) suggest that reducing meat consumption on a global scale would meet with considerable opposition, as consumers who regularly consume meat are unlikely to change their dietary habits. However, a recent analysis by Säll and Gren (2015) demonstrated that imposing a tax on meat and dairy products could potentially reduce environmental impacts of Swedish livestock production by 12%.

The average UK inhabitant consumes 79 kg of meat per year (AHDB, 2013), and meat plus dairy production is cited as contributing ~6% to annual UK GHG emissions (Department of Energy and Climate Change, 2016). To take the simplistic view that a one-day per week reduction in meat consumption would cut animal production by one-seventh, if every one of the UK's 65 million inhabitants adopted such a dietary change, the projected annual reduction in national GHG emissions would be less than 1%. Moreover, livestock production does not simply produce animal-source foods, but also multifarious by-products, including hides, fertilizer, tallow and pharmaceuticals. It is not yet clear what environmental impacts would result from replacing manure with inorganic fertilizers; pharmaceutical ingredients with synthetic alternatives; or from the disposal of the many by-products from human food and fibre production currently consumed by livestock (e.g. sugar beet pulp, maize gluten, cottonseed meal). Gill (1999) estimated that 37 kg of by-product feeds suitable for livestock are produced from every 100 kg of plants grown for human food.

The relatively low feed conversion efficiency of plant-based feedstuffs into animal proteins is likely to remain one of the biggest arguments against the omnivorous diet; yet is also one of the major advantages of livestock production, specifically production of high-quality proteins from human-inedible forages within ruminant systems. When comparing resource use for various human diets, Fairlie (2010) noted that converting from a conventional omnivorous diet to a vegan diet would reduce overall land use, yet the reduction was almost entirely confined to pasture land used for grazing. The quantity of land used to produce annual crops was increased in Fairlie's vegan scenario both due to the lack of animal manures for fertiliser (therefore lower crop yields) and the need to provide vegetable-based fats and oils for energy within the

human diet. Globally, over 3 billion ha of pastureland are currently used to raise livestock, yet only a small proportion of grazed pasturelands are suitable for food crop production due to terrain, water or nutrient restrictions.

Consumer Perceptions of Sustainability

Although survey data overwhelmingly concludes that price, taste, convenience and nutrition are the major factors affecting purchasing decisions (Simmons, 2009; Vermeir & Verbeke, 2006), these are still dependent on the product being morally or ethically acceptable to the consumer. Greater emphasis is being placed on non-tangible attributes such as “environmentally-friendly” or “natural” foods – indeed Robinson and Smith (2002) reported that 52% of consumers showed interest in purchasing “earth-sustainable foods”, and White and Brady (2013) reported that consumers would pay a 4% premium for beef products perceived to have positive effects on environmental impact, which increased to an 82% premium for products with a combined environmental and health benefit. However, cited interests only translate into a change in buying behaviours for a small segment of consumers, hence the relatively small market share for niche systems such as organic, local or natural foods at present (Vermeir & Verbeke, 2006).

Maintaining and improving sustainability in the face of a consumer market that is increasingly skeptical about food production transparency and management practices is a considerable challenge. According to a recent report from SHS FoodThink (2013), only 22% of consumers believe that the agricultural industry is transparent about their practices, yet 53% of consumers trust farmers (compared to academics at 51%, media at 21% and politicians at 8%). Therefore the livestock industry has a considerable opportunity to explain management systems and practices to the consumer, in order to improve transparency and the social acceptability of milk, meat and eggs (Capper and Yancey, 2015). The range of improvement opportunities available indicate that the future livestock industry has the potential to be sustainable, providing that a concerted effort to improve made by all sectors within the supply chain.

Conclusion

Implementation of management practices that improve productivity, reduce resource use and GHG emissions; improve economic viability and maintain the supply of affordable milk, meat and eggs to the consumer; must be implemented with due consideration for economics, market and cultural forces. Mechanisms and programs to better educate the consumer, either directly or through retail and media channels will be essential to attempt to allow for a better understanding of livestock production and reduce the risk of animal-source foods being replaced by plant-based proteins within the human diet.

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Culicoides biting midges and bluetongue virus transmission: What have we learnt since 2006?

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Despite *Culicoides* biting midges being among the smallest blood-sucking flies, with body lengths that rarely exceed three millimeters, they are responsible for the transmission of several internationally important viruses of livestock and horses. As true flies, the developmental cycle of *Culicoides* consists of egg, four larval instars, pupa and adult and the immature stages in particular require moisture-rich habitats. The availability of these environments is therefore a key factor limiting their distribution, abundance and seasonal occurrence. Almost 1400 extant and extinct species of *Culicoides* have been described from a highly diverse range of ecosystems and the genus is present on all major land masses with the exception of Antarctica and New Zealand. Only an extremely small proportion of *Culicoides* species have a significant deleterious impact on human existence and these are generally those species that are effective in taking advantage of environmental changes implemented by agriculture. As a genus, they remain among the least studied of the major insect vector groups. This is in part a consequence of their small size and fragility, which imposes significant limitations on studies of their biology and prevents laboratory colonization of the vast majority of vector species, but is also due to the limited direct impact of *Culicoides* on human health in comparison to other vector groups (e.g. mosquitoes).

Female adult *Culicoides* act as biological vectors, meaning that there is a requirement for the viruses they transmit to infect, replicate and disseminate within them, passing from initial ingestion of blood from viraemic hosts through the gut and into the salivary glands, whereupon transmission can occur. These arboviruses (arthropod-borne viruses) are hence reliant in distribution on the presence of both species and populations of *Culicoides* that can transmit them and susceptible hosts. Globally, the most important arbovirus transmitted by *Culicoides* at present is bluetongue virus (BTV), which can replicate in all ruminants examined to date and is the aetiological agent of the haemorrhagic disease bluetongue (BT). Multiple and persistent incursions of BTV strains into Europe continue to have a huge socioeconomic impact both directly through clinical BT and indirectly through those livestock movement restrictions employed to limit BTV spread. *Culicoides* also act as vectors of African horse sickness virus (AHSV), which causes African horse sickness (AHS), a lethal disease of horses. While currently confined to Africa, AHSV remains a threat to Europe and imposes strict restrictions in the movement of horses from this region to other countries. Additional *Culicoides*-borne arboviruses of notable applied interest include Schmallenberg virus (SBV), a novel virus discovered in 2011 in Germany that causes reproductive problems in cattle and epizootic hemorrhagic disease virus (EHDV) which has a major economic impact on deer production in the USA.

During the last two decades there were significant changes in the global epidemiology of *Culicoides*-borne viruses. These were most notable in Europe, which experienced outbreaks of unprecedented severity and persistence. As a result, a disproportionate number of studies of *Culicoides* have been carried out in this region and we will mostly focus on this region in describing recent research. Following the initiation of BTV outbreaks in 1998, studies were initially based in the Mediterranean basin, but following the incursion of the incursion of the BTV serotype 8 (BTV-8) into the Netherlands during 2006 focused increasingly on North-Western Europe. We will examine how novel techniques in genetics, genomics and mathematical modeling were used by a rapidly expanding community of entomologists studying *Culicoides* to both inform responses to the virus incursions and to understand the primary drivers of transmission. We will also examine studies from outside Europe which allowed a broadening of the range of techniques used to examine the *Culicoides* genus and novel approaches devised for this genus.

The most obvious entomological response to *Culicoides*-borne arbovirus outbreaks across Europe has been the implementation of wide-scale surveys of abundance of adult midges, including identification of geographic and temporal incidence. This has been developed through wide-scale and highly intensive trapping campaigns in Italy, Spain and France in particular. Surveys use light-suction traps to record the numbers and species of *Culicoides* collected, but are prone to a wide range of biases, not least variation in how attracted species are to these baits. Nevertheless, as a relative means of monitoring abundance these traps provide a logistically straightforward solution and have proved to be very popular as research tools. The main reason for funding this surveillance is to obtain data enabling demarcation of risk of arbovirus transmission in space and time. These estimates of risk need to be sufficiently robust, however, to be of utility in drafting legislation to enable safe movement of ruminants (through identification of periods of adult vector activity) or to drive vaccination campaigns.

Data regarding species abundance is underpinned by a taxonomic framework of varying quality which is usually almost entirely focused on livestock holdings. *Culicoides* can be separated morphologically by their wing patterns and several other traits, however, overlap of characteristics and a lack of dedicated expertise has been a major barrier for research on the genus. It is known that *Culicoides* vary in their ability to act as vectors of arboviruses according to species and population, influencing the spread and persistence of these viruses. As an example, vector competence for BTV (the proportion of the population able to transmit the virus) varies at both a species and population level. Variation in vector competence is thought to determine BTV distribution in the eastern states of the USA via the presence or absence of confirmed vectors. Modeling of the spatial and temporal incidence of adult *Culicoides* has played a major role in attempts to understand the drivers underlying the changing epidemiology of BTV in Europe. The influence of climate change on the transmission of BTV has provided a major point of debate, brought into focus by influential publications that have integrated mathematical modeling, entomology and virology. At least one aspect of this debate has been settled in the form of the hypothesized recent geographic spread of vector species, but alternative regional-scale hypotheses to explain the dramatic shift in BTV epidemiology that do not invoke climate change have not arisen. In this regard, an understanding of transmission parameters for *Culicoides* to spread arboviruses might be of specific interest.

In Europe, risk of transmission for BTV, AHSV and EHDV was for several decades related directly to the presence or absence of *Culicoides imicola*, a primarily Afrotropical species which has a vast global distribution reaching from the Republic of South Africa to Spain and India. This species had been implicated convincingly in the transmission of arboviruses in both field and laboratory based studies. Naturally, following major incursions of BTV into mainland Europe from 1998 onwards, this species was the main suspect for transmission and surveys concentrated upon monitoring *Culicoides imicola*'s range in this region using light-suction trap surveys across the Mediterranean basin and southern Europe. It became increasingly obvious, however that other vector species were involved in transmission of BTV strains within areas where *Culicoides imicola* were absent, leading to concerns regarding expansion of the virus into northern Europe.

These fears were realised when BTV-8 emerged in northern Europe during 2006. In this region entomologists faced a significant problem in separating species that might be involved in transmission using their morphology and polymerase chain reaction (PCR) based methods were implemented into identification. It is notable, however that despite the huge economic impact of BTV in Europe since the late 1990's, attempts to characterize the *Culicoides* fauna within European countries have been very small scale in comparison to funds spent on wider surveillance networks. This matters due to the fact that these species of *Culicoides* are known to vary significantly in their ecology, relationships with hosts and seasonal and geographic incidence. This can have major implications

where attempts are made to model Culicoides as a single taxonomic unit and then predict biological outcomes due to the fact that multiple species with different responses are likely to be conflated in analyses. While genetic analysis studies in Europe were broadly congruent with the morphological framework used to identify possible vectors, additional species were also proposed which have not since been integrated into standard surveillance reporting.

Despite these challenges, significant progress has been made in northern Europe in linking species identification of Culicoides to their ability to transmit viruses in the field and laboratory. These studies illustrate both the importance of understanding the process of arbovirus infection in Culicoides and also the advantages that emerging technologies provide. Early attempts to implicate species in transmission of BTV-8 were plagued by the misunderstanding that detection of RNA in specimens was indicative of infection. Quantification in such studies is vital as RNA is known to persist both in Culicoides that take up virus in their blood meal but don't become infected and in those that become sub-transmissibly infected (i.e. where the virus does not disseminate to the salivary glands). Later experimentation, however, integrated robot-based technology and real-time RT-PCR into screening of field collected Culicoides for BTV RNA, enabling unprecedented speed of processing and quantification. The use of real-time RT-PCR to detect virus is also far more robust than isolation of arboviruses in not requiring a cold chain and is more easily paired with ethanol-based preservation which is used by the vast majority of collectors. Recent studies in the Netherlands directly linked disseminated SBV infections to molecular marker sequences in Culicoides. These studies are now being extended further using next generation sequencing technologies that are likely to revolutionize such studies, particularly in areas of endemic transmission. A key area that will be explored within this area is the impact of co-infection of Culicoides which may influence vector competence alongside genetic determinants.

Transmission of BTV in many other regions of the world remains poorly characterized, with either no or limited modern information available for entire regions including South America, India and China. This is largely a consequence of either the limited impact of Culicoides-borne arbovirus circulation in these regions and logistical difficulties in field-based research. With the anticipated availability of PCR-based technologies for research in this area in laboratories worldwide, studies of vector competence in Culicoides in the field and laboratory should begin to make a truly global understanding of virus: vector interactions achievable. This is important in understanding the impact of global trade on arbovirus transmission which to date is poorly understood for Culicoides-borne pathogens.

Another consequence of the development of PCR based assays has been the ability to detect blood-meal origin in Culicoides as a means of understanding what animals they prefer to feed upon. Most of the Culicoides species responsible for arbovirus transmission in Europe have been found to utilize a relatively wide range of mammalian hosts that often includes human beings. Beyond these species, there are distinct differences between Culicoides that feed on mammals and those that feed on birds that remain poorly investigated. In particular, the cues used by Culicoides to find their hosts remain largely unknown. Studies of blood-meal origin provide limited information where they are not paired with detailed descriptions of host availability and ecology in the environment (e.g. if you collect Culicoides from close proximity to cattle it may be very probable they will have fed on cattle).

True manipulative studies of Culicoides host-preference, where insects are collected on animals to define which species are feeding on them and when remain extremely rare. This is largely due to the major effort that is required to conduct them and also the fact that studies tend to be highly specific to the site and host combination used. An area that requires investigation in BTV epidemiology is Culicoides host-preference between adult cattle and sheep. There is a large amount of anecdotal evidence that cattle attract greater numbers of BTV vector Culicoides and hence may act to drive BTV transmission. Co-grazing sheep flocks with cattle

herds may have an impact in increasing or decreasing Culicoides biting rates. Culicoides host preference for individual animals, which has been characterized for Culicoides and human beings in Scotland, has also not been demonstrated for livestock. This could be approached through the use of rapidly evolving molecular markers for hosts that would allow tracing of blood-meals to a specific individual.

Another advance has been the implementation of techniques based on genomics, although these are currently only applied to colony lines of the North American vector Culicoides sonorensis. Transcriptomic analyses have already been applied to examine the areas of developmental biology in Culicoides sonorensis and aspects of innate immunity. In addition, the first full genome construction for any Culicoides has been initiated and is due for public release by The Pirbright Institute and the European Bioinformatics Institute during 2016. These studies are likely to provide a major focus for studies in the upcoming decade and a wealth of opportunities for collaboration between entomologists, geneticists and bioinformaticians. While studies at present are entirely focused on colony lines of Culicoides sonorensis, the upcoming decade is likely to see such techniques applied increasingly to other species.

Among candidate species, Culicoides imicola would be an obvious target for study as it possesses a vast geographic range and has a huge global impact as a vector of arboviruses. Methods to blood-feed Culicoides imicola have been developed that could enable genome-level analysis of vector competence. In addition, progress has been made in colonizing this species although although several aspects remain challenging. Productive colonies of Culicoides imicola would enable areas such as vector competence, immunological responses and physiological analyses to be approached more easily. They would also assist in the production of cell lines specific to this species to complement those already produced for Culicoides sonorensis. Among the other primary vectors of BTV worldwide, there are several major challenges to be overcome before the full potential of genomic techniques can be realized. For many species, including all of those acting as major vectors of BTV in Europe with the exception of Culicoides imicola, blood-feeding techniques are only poorly effective and all previous attempts at colonization have failed.

Within what is a relatively small community, a major determining factor in how successfully we implement techniques based on genetics, genomics and mathematical modeling will be in connectivity between workers. Through the almost universal use of internet-based resources by scientists, communication between veterinary workers, virologists and entomologists has advanced hugely in the past decade and demarcation between these roles have blurred in some cases. The increasing use of free-access resources will become commonplace as alternatives to traditional publishing, alongside broader data repositories. It is likely that public funding agencies will also increasingly demand public accessibility of datasets from across research areas as part of their support and this may improve the current sharing of information. This may improve the continuity of datasets which at present are fragmented across countries and regions, in part due to the way in which funding is obtained.

A reverse vaccinology approach to developing a vaccine for digital dermatitis.

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Digital dermatitis (DD) is a major disease of dairy cattle which presents as severely inflamed lesions on the rear feet. The lesions are extremely painful, and are associated with significant lameness making DD an important animal health concern and one of the most important infectious diseases of cattle. Thus, DD is a very important animal welfare issue which has serious economic implications for the dairy industry in the UK and worldwide. In the UK and USA, DD is considered endemic on dairy farms; it has substantial financial implications and is considered second only to mastitis as a concern for farmers. Furthermore, Bovine DD (BDD) is an important worldwide food security issue as it results in reduction in milk yield and reproductive performance. Specifically, BDD is estimated to cost a dairy holding of 65 cows \$1,517/year which equates to costing the UK dairy industry (1.85 million dairy cows) a considerable £26.4 million/year. Clearly, DD has a negative effect on UK economy where milk accounts for 16.1% of UK total agricultural output.

In recent years, DD has crossed species barriers and is now commonly reported in sheep (UK and Eire) where it is classified as a rapidly emerging disease. Furthermore, DD is increasingly found in beef cattle and is now also reported in UK goats, with very serious clinical consequences. In each species, available prevention and treatment measures fail to control DD spread and associated disease outcomes; with the disease frequently reappearing. Recently, we have highlighted the potential role of foot trimmers in disease spread.

The infectious nature of the disease is apparent and it is now widely accepted that the primary infection in each host species is the novel spirochetal bacteria. These are found in large numbers in all BDD cases, often deep within foot lesions. Molecular methods have further identified these spirochetes as *Treponema*. Cloning of bacterial 16S rRNA genes indicated 5 phylotypes of treponemes in BDD lesions from Germany. This has led to the isolation and characterisation of three of these five treponeme groups from lesions in USA and UK. Recent molecular epidemiology work at the UoL and elsewhere has identified treponemal bacteria as the common causative agents. Furthermore, an infection model recently used pure cultures of treponemes applied to the bovine host feet; the results close to fulfilling Koch's postulates to establishing a causal link between DD and treponemes.

Hence, it is imperative that a different (and preferably improved) disease management approach is needed in the near future; a vaccine that prevents the primary treponeme infection would be both ideal and timely, especially when used in conjunction with other on-farm approaches to improve hygiene and biosecurity. Such a vaccine would probably be applicable to the other known DD host species of beef cattle, sheep and goats. It may also be valuable for prevention of the many newly reported skin lesions (in several species, including pigs and horses and wild deer) associated with DD treponeme colonisation.

Prevention, control or eradication of digital dermatitis – developing a vaccine.

Whilst perfect hygiene cannot be readily achieved on dairy farms, it is clear that well managed farms have lower incidence, prevalence and severity of DD in their herds. Dairy cow DD is typically treated with footbaths of toxic compounds (such as formalin or copper sulphate) or topical antimicrobial agents which are both relatively ineffective, as lesions soon reappear. These current treatments are considered moderately effective at killing the secondary invasive bacteria, but have little effect on the deeper lying treponemes, the primary infection and thus, the disease readily recurs. Also, and very importantly, both

current treatment options are being legislated against within the EU (and potentially wider) due to either environmental risks or fear of induction of antimicrobial resistance in farmed species. Thus, prevention of disease transmission or an effective vaccine would be better options.

As the only consistent bacterial presence in DD lesions is the specifically associated treponemes, it is apparent that targeting these bacteria would be the best chance to control disease. A bacterin vaccine based on a single BDD treponeme phylogroup was initially reported to reduce the incidence of BDD but subsequent studies reported the vaccine as ineffective. As most lesions show co-infection by several BDD treponeme phylogroups, a multivalent vaccine containing cell surface proteins from several of these BDD treponeme phylogroups may finally allow for effective control of this debilitating disease. As some phylogroups of BDD treponemes are not yet cultured, identifying antigens shared by the three isolated phylotypes should allow for future vaccines to include further recombinant antigens, including proteins encoded from genes directly mined from lesions, which would bypass problems associated with growing these fastidious microbes.

Targeting treponemes to control digital dermatitis – scientific case.

Whilst DD is undoubtedly a polymicrobial disease, there is little dispute that treponemal species are the only consistent infection in all cases and that they are the probable initiating infection, with their being little evidence for consistent primary contribution of other micro-organisms.

Research, mainly at UoL, has resulted in protocols (immuno-magnetic separation/ selective media) to isolate three groups of treponemes from dairy cattle DD lesions. As a result, the UoL team now have a large treponeme isolate archive of over 100 treponemes from DD lesions as well as more than 20 from various animal tissues and, particularly, from the skin and gastrointestinal tract. Subsequently, we have generated complete genomes for representatives of these three DD-associated phylogroups as well as commensal bovine GI tract treponemes and closely related human equivalents. Collectively, this is an excellent research resource and has enabled many key developments towards a pathway for controlling this disease.

Characterisation of these spirochetes identified the isolated BDD treponemes into three groups: *Treponema* medium-like, *Treponema* phagedenis-like and *Treponema* denticola-like spirochetes, respectively, based on similarities with human oral and urinary tract treponemes as determined by 16S rRNA and flagellin gene sequencing and phenotypic characterisations. Importantly, these spirochetes, although in three distinct groupings, still cluster tightly together and are very separate from those isolated from cattle GI tract contents and from other host species and diseases. Further characterisation of the *T. denticola*-like BDD spirochetes allowed them to be designated as a new species - *Treponema* pedis. Subsequent studies demonstrated the susceptibility of BDD treponemes to a number of relevant antimicrobials with a view to potential usage in vivo. Development of PCR assays for detection of the three groups of isolated BDD treponemes then identified a high association for all three groups in UK BDD lesions. This corresponds to a recent BDD fluorescence in situ hybridisation study that identified (but did not isolate) five groups as involved in BDD aetiology, with three groups resembling treponemes isolated at UoL.

Hypothesis and approach

The central hypothesis is that by combining genomic data with novel in silico and in vitro technologies, we can identify, generate, characterise and deliver surface expressed treponemal molecules as vaccine candidates to prevent and/or control digital dermatitis in dairy cattle. If successful, the respective outcomes would enable large scale trials and potential production of a vaccine to significantly improve animal welfare, farm economics and food security.

Aims and objectives

- Using genomes and proteomics, identify surface expressed treponemal proteins which have potential as antigens and vaccine components.
- Expression, purification and reconstitution of recombinant treponeme proteins from *Escherichia coli*.
- Identify functional and virulence activities of recombinant treponeme proteins.
- Testing immunogenicity of candidate vaccine antigens in vivo
- Efficacy trials of selected treponeme proteins to test ability to prevent DD lesions developing.

Reverse vaccinology approach

Reverse vaccinology (RV) is a relatively new concept and describes the process of candidate vaccine target selection that begins with the genome of the pathogen rather than the pathogen itself. The entire genetic (and therefore antigenic) potential of the infectious agent can be scrutinised. This method permits not only the potential identification of all antigens that would otherwise have been discovered by conventional approaches, but also novel antigens not detectable or overlooked by traditional methods. In addition, reverse vaccinology is a relatively fast process (1-2 years), able to identify virtually all antigens, regardless of time of expression, abundance or immunogenicity.

Using bioinformatics approaches, the genome can be screened in silico for genes that encode proteins with antigenic potential. A computational approach to the subcellular localisation of bacterial proteins, although historically imprecise, now compares favourably to high-throughput laboratory approaches, with a wide range of bioinformatic tools freely available online for this purpose.

Genomes and bioinformatics.

From the Liverpool DD treponeme collection, genomes were generated (Roche 454 sequencing) from representatives of the 3 cultivable DD treponeme phylogroups and from commensal cattle treponemes. In silico analysis of the genomes enabled comparative genomic selection to identify genes coding for homologues of proteins likely to be surface expressed (SignalP). Once a protein's subcellular localisation is established, protein topology prediction is an important consideration during the in silico vaccine candidate selection process. Crucially, when attempting to predict prokaryote outer membrane proteins from an amino acid sequence, discrimination must be made between the integral α -helical proteins that predominate in the cytoplasmic membrane and the integral β -barrel proteins that predominate in the outer membrane. All structurally-characterised gram-negative bacterial transmembrane OMPs to date have been found to share essentially the same β -barrel topology, consisting of 8-22 anti-parallel β -barrel strands folding into a cylindrical β -barrel structure (TM-BETA, PRED-TMB). These β -barrels were prioritised in our bioinformatics search. Consequently, a large panel of putative surface expressed antigenic proteins was produced for each DD treponeme and homologs identified across phylogroups. The resulting proteins were all hypothetical and without predicted function.

Production of recombinant treponeme proteins.

Genes for selected proteins were cloned into an entry vector (pENTR), transferred into an expression vector (pDEST) and expressed in *E coli* (BL21 DE3), eventually in large scale cultures. Recombinant proteins were purified by metal affinity chromatography and refolded to native structures as assessed by SDS-PAGE and CD spectroscopy. To date 33 recombinant DD Treponeme proteins have been cloned expressed purified and successfully refolded. These have then been assessed for biofunctions such as antigenicity and evidence of virulence potential.

Antigenicity of the recombinant treponeme proteins.

Sera from cattle with and without DD lesions were screened for reactivity to recombinant treponeme proteins by ELISA and Western blotting. Many were not detected as antigenic in naturally occurring disease.

Function of recombinant treponeme proteins

Bio-assays were developed to assess the ability of recombinant treponeme proteins to bind to cattle connective tissue molecules as this would be important in pathogenesis. These highlighted those likely to attach well to host tissues and hence potential key vaccine targets.

Immunogenicity studies.

Currently, we are testing a short list of recombinant treponeme proteins (selected from the above antigenicity and bioassays) in calves to identify any generating strong immunity. Any successful proteins will then be taken forward into efficacy trials.

Conclusions

Reverse vaccinology is a very powerful tool to rapidly generate novel vaccine targets, especially for organisms for which there is little available biological data.

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Placing helminth infections of cattle in an economic context

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Abstract:

Helminth infections of cattle affect productivity in all classes of stock, and are amongst the most important production-limiting diseases of grazing ruminants. Over the last decade, there has been a shift in focus in the diagnosis of these infections from merely detecting presence/absence of infection towards detecting its impact on production. This has been facilitated by studies observing consistent negative correlations between helminth diagnostic test results and measures of productivity. Veterinarians are increasingly challenged to consider the economic aspects of their work, and the use of these tests should now be integrated in economic evaluation frameworks for improved decision making. In this paper, we review recent insights in the farm-specific economic impact of helminth infections on (dairy) cattle farms as well as in farmer attitudes and behaviour regarding helminth control. Combining better economic impact assessments of helminth infections together with a deeper understanding of the non-economic factors that drive a farmer's animal health decisions should result in more effective control strategies and increased farmer satisfaction.

Key words: Cattle, helminth, diagnosis, nematode, liver fluke, helminth, economics, communication

Introduction

Cattle are parasitized by various helminth species, the most important being gastrointestinal nematodes (GIN), lungworms and liver fluke. These pathogens can cause severe disease, affect productivity in all classes of stock, and are amongst the most important production-limiting diseases of grazing ruminants. Essentially all herds/flocks in a grass-based production system are affected. Infections with GIN and liver fluke are more chronic and the major economic impact is due to sub-clinical infections causing reduced growth, milk yield and fertility (Morgan et al., 2013). Infections with lungworm are more acute and can place a sudden high economic burden on a farm due to mortalities and sharp decreases in milk yield (Holzhauer et al., 2011). A recent comprehensive study, funded by the EU-GLOWORM project suggested an annual loss in the EU dairy cattle industry of €2.3b from GIN infections alone (P. Torgerson, personal communication).

Over the last decade, the pressure on farm income has further increased due to higher production costs and fluctuating output prices (Thornton, 2010). Subtle changes in production efficiency can make the difference between profit and loss. Efficient farming with an optimal management of inputs such as stock, feed, and labour has therefore become increasingly important. Animal health decisions have a significant impact on production efficiency, but are also subject to resource scarcity and budget constraints. Veterinarians are thus increasingly challenged to consider the economic aspects of their work for a farmer. Hence, economic evaluation frameworks are needed that can be integrated in decision making.

Once the economic value of specific animal health interventions can be demonstrated, veterinarians are faced with a second problem. How can they convince the farmer to implement their advice? Often the claim of an economic benefit will not be sufficient to induce a real change in

farm management, even if it is grounded on solid scientific evidence. In other words, we need to understand the complete rationality of farmers' behaviour in order to improve compliance with the provided advice (Garforth, 2015).

In recent years, research in these 2 fields (i.e. economics and socio-psychology) is emerging in the field of animal health in general as well as in the field of helminth control in ruminants. In this paper, we discuss the diagnostic tools and methods that are available to assess the economic impact of helminth infections on (dairy) cattle farms as well as recent insights in farmer attitudes and behaviour that can help in the development of effective communication strategies to increase the uptake of proposed intervention strategies.

Diagnosis to assess production impact

Over the last decade, an important paradigm shift occurred in the diagnosis of parasitic helminth infections in cattle. There has been a shift in focus from merely detecting presence/absence of infection towards detecting its impact on production. This is important because i) helminth infections are highly prevalent and ii) not every infection is of economic relevance. For example, fasciolosis is mostly a chronic disease, and often it is already when the infection is around on the farm. Instead of demonstrating presence of the infection, it is more relevant to identify the associated production losses to convince farmers that further diagnosis, and control measures, are worth considering (Charlier et al., 2014c). This paradigm shift has been made possible by epidemiological research that observed consistent negative correlations between helminth diagnostic test results and measures of productivity.

In first-season grazing cattle, the serum pepsinogen concentration can be used to discriminate between different levels of *Ostertagia ostertagi*-infection and morbidity and associated production losses (Dorny et al., 1999). However, the lack of standardization between laboratories and relatively high cost of the test are important constraints to its widespread uptake (Charlier et al., 2011). Faecal egg counts (FECs) of GIN correlate well with initial infection rates approximately 2 months after turnout on pasture for first-season grazing animals. However, after that period, host immunity reduces the correlation with actual worm burden and it seems impossible to indicate what weight gains are obtained by the end of the first grazing season, from FECs measured early in the season (Shaw et al., 1998). Therefore, FECs are primarily considered useful for understanding epidemiology rather than assessing infection levels or production impact (Sargison, 2013). This picture could change with new FEC techniques such as FLOTAC that detect helminth eggs in faecal samples with unprecedented sensitivity (Cringoli et al., 2010).

In adult cattle, consistent negative relationships have been demonstrated between antibody levels to GIN or liver fluke in bulk tank milk and herd-average milk production (Charlier et al., 2005; Mezo et al., 2011; Sanchez and Dohoo, 2002). In beef cattle, the quantification of antibody levels against GIN and liver fluke in meat juice obtained in the abattoir has been proposed, showing negative correlations with carcass weight and conformation score (Charlier et al., 2009). Studies have also shown negative relationships between helminth-specific antibody levels and reproduction and mortality indices at the herd level (Delafosse, 2013; Sanchez et al., 2002). Most recently, negative correlations have been established between bulk tank milk ELISA for lungworm infection and milk production. Results showed a difference in milk production and milk fat of 1.0-1.7 kg/cow per day and 0.08-0.14 % between lungworm positive and negative herds, respectively (Dank et al., 2015), providing for the first time evidence of the economic importance of subclinical lungworm infections.

These established relationships can be used to indicate helminth-induced production losses associated with a test result of a specific farm (Charlier et al., 2012). Several limitations with this approach remain, such as the lack of species-specificity of the measured antibody levels and the rather weak relationship between detected antibody levels

and production responses after anthelmintic treatment. Nonetheless, it provides an ally to communicate to farmers on the importance (or not) of a helminth infection and to help monitor potential production losses (Charlier et al., 2014b).

From production to economic impact

The impact of helminths on animal productivity is increasingly well understood (Charlier et al., 2014b), but the economic impact depends on multiple other factors such as farm-specific input and output prices and local regulations. The established links between diagnostics for different helminth infections with production losses, now allow to include this information in models that aim to assess the economic impact of the infection at regional or even farm-level. Such economic models of animal diseases are important because they contribute to balance expenditures on disease control with the actual disease costs and to evaluate the economic attractiveness of animal health interventions compared to other investment opportunities (Yalcin et al., 1999).

Considerable progress has been made in recent years with models to estimate the cost of helminth infections and/or interventions measures at the farm level. Some of these models are available to veterinarians at www.ParaCalc.com. First, there is a deterministic spread-sheet model ("cost of worm infections") where results from diagnostic methods (i.e. pepsinogen assay and serum ELISA for growing cattle and bulk tank milk ELISAs for adult cattle) to monitor the helminth infection status on a dairy herd and anthelmintic usage are used as input parameters. It produces a report with the expected annual loss due to infections with GIN and liver fluke to discuss with the farmer. The model is useful to evaluate the general importance of the infections, to monitor the evolution of costs across different years and to benchmark the results with peers. However, it does not consider the principal of "recoverable loss" (Perry and Randolph, 1999). How much of the total costs induced by helminth infections can be avoided by intervention measures? This is often difficult to determine because: (i) it is impossible to eliminate the infection from a farm; (ii) there can be remaining tissue damage after effective treatment or (iii) re-infection can occur at varying levels. Obtaining such information requires the set-up of experiments, by preference under commercial farming conditions that evaluate the impact of specific intervention measures. Such information is increasingly available, especially to evaluate the production effects of strategic anthelmintic usage (e.g. Höglund et al., 2013; Verschave et al., 2014, and this information was used to develop the second tool "treatment strategies against gastrointestinal worms" on ParaCalc.com. It estimates the likely economic benefit and uncertainty of a number of anthelmintic treatment strategies of adult cows and produces a report to discuss with the farmer.

Most recently, the farm-specific economic impact of helminth infections has also been studied using efficiency analysis (van der Voort et al., 2013). Efficiency analysis studies the conversion of input(s) into output(s) and compares the current performance level of a farm with the performance level of peer farms with similar production technologies (Coelli et al., 2005). Using this approach, GIN infections appeared to mainly constrain the efficient transformation of pasture, health related costs and labour into milk. The inefficiency related with GIN infections was reduced when higher levels of concentrates and roughage were supplied (van der Voort et al., 2014). Efficiency analysis has the potential to identify different improvement paths depending on the farm-specific production process and this was recently investigated by van der Voort et al. (2016). Farms were clustered in 3 groups depending on technical efficiency (TE) and input use. In low TE farms with a relatively low use of concentrates, there was no correlation between TE and level of exposure to GIN. Therefore, they are unlikely to improve economic performance by lowering the exposure to GIN infections. Analysis suggested they could best improve economic performance by making more use of concentrates. In farms with an intermediate TE and relatively high use of concentrates, there was a strong correlation between GIN exposure and TE. In addition, analysis showed that economic performance could

be improved by substituting part of concentrates by grazing, which could lead to a higher infection pressure. This makes monitoring GIN infection crucial in this group. In farms with the highest TE and intermediate use of concentrates, there was also a negative correlation between TE and GIN exposure. Analysis showed that economic performance could be improved by both reducing input use and reducing infection. In conclusion, efficiency analysis allows to establish links between animal disease and input use and input transformation. It can detect trade-offs and synergies between animal health interventions and input-output transformation. Whereas the implications of a vet's advice are traditionally restrained to animal health issues and the improvement of technical key performance indicators, with this technique, we should be able to place our advice better in the whole-farm economic context. This approach is still in the research phase, but it is to be expected that it will be integrated in practical decision support tools for herd health management in the medium term.

Non-economic factors that drive animal health decisions

Suppose that we have a high quality economic assessment report at hand to discuss with the farmer and that we are able to distil clear suggestions to improve his animal health management. Will this be sufficient for the farmer to implement our proposed strategies? It is now well understood that farmer's decisions about their enterprises are not solely based on financial and business criteria. Farmer's motives are rooted in deeply held values and also influenced by attitudes, beliefs and social norms (Garforth, 2015). Understanding all the values that drive farmer behaviour requires socio-psychological research, aimed to lead to increased understanding of a farmer's rationality and more effective advisory interventions (Charlier et al., 2015; Garforth, 2015).

In the field of helminth control, Vande Velde et al. (2015) investigated farmers' intention to adopt diagnostic methods before implementing anthelmintic drugs in cattle. Based on two fundamental theories in the fields of behavioural and health psychology, a survey was carried out in 574 Flemish dairy farms to investigate the influence of the following variables: 'attitude towards preventive use of anthelmintics', 'attitude towards diagnostic tools', 'subjective norms', 'behavioural control' and 'perceived risk'. The results showed that 'attitude towards diagnostic methods' and 'subjective norms'; i.e. the influence of significant others, had the strongest, positive influence on adoption intention of diagnostic methods. 'Attitude towards the preventive use of anthelmintic drugs' had a negative effect on adoption intention of the diagnostic methods. 'Perceived risk', which was defined as the perceived susceptibility and severity of anthelmintic resistance on their farm, had no effect on the intention to adopt diagnostic methods. These results implicate that if we want to persuade farmers to make more use of diagnosis before anthelmintic treatment decisions are made, we should reinforce their positive attitude towards diagnosis and make use of their social network, which could implicate family, peer-farmers and the veterinarian. At present, the argument of anthelmintic resistance has no or little effect on dairy farmers' intention to use diagnostics for helminth diseases.

In order to investigate how veterinarians can improve their communication, we can learn from socio-psychological studies that have been conducted on different topics such as biosecurity, notification of notifiable diseases, antimicrobial usage and mastitis management (Delgado et al., 2012; Hamilton et al., 2016; Jansen et al., 2009). Although the results cannot be extrapolated beyond their scope, similar patterns often emerge. Identification of different behavioural types is a first step towards more adapted advices and increased compliance. In the UK, Rehman et al. (2008) differentiated farmers with a family orientation from entrepreneurs, life-stylers, hobbyists and independent farmers, respectively. In Brazil, Pereira et al. (2016) were able to classify beef farmers that were considered receptive towards novel technology adoption based on their main sets of goals and values: the professional farmer, the committed environmentalist, the profit maximiser and the aspirant top farmer. The study showed a considerable diversity of values and goals even within

this subset of progressive farmers. This diversity should be taken into account, because the advices that are in accordance and reinforce the farmer's core values will have the highest uptake. Age may also be an important criterion as Hamilton et al. (2016) showed that young farmers (< 45 years) tend to be more entrepreneurial and amenable to change. Finally, the subjective norms, i.e. the social network surrounding the farmer, is often one of the most influential factors in driving animal health decisions (Garforth, 2015; Jones et al., 2015). This can be exploited by the organization of farmer groups to provide a forum where farmers can explore management options and learn from each other's views and experience (Vaarst et al., 2007). Group learning is most successful if it includes experiential learning, group autonomy and builds on ongoing relationships and learning opportunities (Millar, 1997). A catch can be that endemic livestock disease can be viewed as a problem for 'bad' farmers and not an issue for those individuals who manage their stock well. As such, there may be a low motivation to form groups to address what is largely perceived as an individual problem (Heffernan et al., 2008).

Conclusion

Several diagnostic tools and methods are now available to assess the economic impact of helminth infections on (dairy) cattle farms. These include herd anamnesis in combination with serum pepsinogen assay and bulk tank milk ELISA for *O. ostertagi*, *F. hepatica* and *D. viviparus*. The use of these diagnostics is being integrated in decision support tools that should allow the veterinarian to estimate the economic consequences of his interventions and advice regarding helminth control. This can contribute in general to a 'diagnosis before treat' approach and thus increase the sustainability of anthelmintic control by a more underpinned and selective treatment (Charlier et al., 2014a).

Besides economic evaluations, more emphasis will have to be put on how advices are most effectively communicated. With the current plethora in websites, blogs and other communication channels, farmers are looking for trustworthy sources where they find reliable information that is fit to their situation. Private veterinarians are widely seen as such credible sources of information (Frössling and Nöremark, 2016; Jones et al., 2015; Richens et al., 2015). Yet it appears that our communicative skills can still be improved. Farmers in general do not voluntarily communicate on their needs regarding animal health (Derks et al., 2013) and, therefore, veterinarians should actively seek those needs. Further, being more explicit during farm visits in discussing the farmer's goals and priorities and providing a clear summary at the end of the visit of any advice given, would mean a significant step forward towards improved veterinary communication (Derks et al., 2013). By understanding the core goals and values of different types of farmers, advices can be better targeted and framed in order to achieve higher compliance and farmer satisfaction.

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Full reference list is available upon request from the corresponding author.

Paratuberculosis

Michael T. Collins

Prophesy fulfilled

"The disease has, at present, a limited number of sources from which it can spread. These sources of infection will continually increase, unless agencies are operative to offset the constantly increasing commerce in cattle from such herds. ...if not controlled, it may become a more troublesome scourge for future generations than tuberculosis is for the present generation of cattle-owners." (V.S. Larson, B.A. Beach & W. Wisnicky J. Amer. Vet. Med. Assoc. 80:446, 1924).

We (veterinary medicine and animal agriculture) had our chance to deal with paratuberculosis before it became an epidemic and we muffed it. In 1900 there were only scattered reports of paratuberculosis. A 1922 Agriculture Experiment Station publication in Wisconsin fully described the disease epidemiology, pathology and methods for diagnosis. It stressed that paratuberculosis (Johne's disease) was seen in a small number of Wisconsin herds urging veterinarians and herd owners to act aggressively to eliminate the disease from infected herds and to protect those that were as yet not affected. Failure to heed these and other such warnings by veterinarians in Europe and the U.S. allowed the disease to insidiously and unrelentingly spread among herds, regions, and countries of the world.

As the famed bacteriologist Stanley Falkow once said: *"The objective of a bacterium is to become bacteria"*. The pathogen causing paratuberculosis, *Mycobacterium avium* subspecies *paratuberculosis* (*M. paratuberculosis*) has evolved very clever strategies allowing it to replicate inside macrophages, circumvent the host immune response, and persist in the environment waiting to be ingested by another susceptible host as a means of steadily increasing its numbers on the planet. Microbiologists have labeled *Mycobacterium tuberculosis* Complex (which includes *Mycobacterium bovis*), the cause of TB, the most successful pathogen on the planet. *M. paratuberculosis* is on track to one day take this title away.

Prevalence today

Today, 121 years since Heinrich Albert Johne described paratuberculosis in a Guernsey cow in Germany, well over half of all dairy herds in virtually all major dairy-producing countries are infected with *M. paratuberculosis*. The estimated herd-level true prevalence in the U.S. is now 91%. This infection, first seen in dairy cattle, has spilled over to cattle of all breeds and types. It also is prevalent in both meat and dairy goats and sheep. In the province of Ontario, Canada over half of sheep and goats herds are infected with *M. paratuberculosis*. Without question, paratuberculosis is a panzootic – and, it just might be a pandemic.

Myriad diagnosis and control tools

Scientists the world over have invested time, effort and millions of dollars toward a better understanding of the pathophysiology of paratuberculosis, its economic impacts, epidemiology, and diagnosis and control measures. Birth of the International Association for Paratuberculosis in 1988 helped foster scientific communication and collaboration. As a result, we bovine veterinarians and paratuberculosis specialists know this disease well and have numerous diagnostic tools at our disposal. We can detect cattle in the more advanced, most infectious, stage of disease using very inexpensive antibody detection tests on serum or milk samples by ELISA technology. We can detect earlier stages of infection as animals just begin fecal shedding of *M. paratuberculosis* using genetic detection assays, commonly called PCR. We have multiple culture methods for isolation of *M. paratuberculosis*, if required. We have sequenced the entire genome of *M. paratuberculosis* and can effectively discriminate among *M. paratuberculosis* strains by an array of genotyping techniques or whole genome sequencing.

We have risk assessment tools that help evaluate *M. paratuberculosis* transmission risks in herds helping to focus herd management changes on those that will limit infection spread the most. At the University of Wisconsin, we have adapted the U.S. dairy herd risk assessment tool to an iPad App facilitating on-farm completion and submission of herd risk assessments. <http://www.vetmed.wisc.edu/dms/fapm/apps.htm>. Typically, herd risk assessments reveal the need for herd owners to focus on blocking fecal-oral infection transmission to calves; a task that improves overall calf health yielding significant pay-offs beyond paratuberculosis control.

Impediments to action

Why then does paratuberculosis continue spreading? Animal trade continues unabated and is the most effective means of infection spread among herds. Barrett et al. (Vet. Rec. April, 2011) showed that the #1 risk factor for dairy herds being found infected with *M. paratuberculosis* was the direct importation of cattle (OR 19.2, p<0.001). Rarely do cattle owners expanding their herds use appropriate pre-purchase infectious disease testing, especially not for paratuberculosis. The result is an ever increasing herd-level prevalence of paratuberculosis across the globe.

Spread of paratuberculosis between countries by animal trade as they enhance their dairy industry has been repeated over and over again. China is the current best example of this. Huge numbers of cattle being imported to China as it rapidly builds a dairy industry. These cattle come from countries well-known to have a high herd-level prevalence of paratuberculosis. The result is that *M. paratuberculosis* becomes added to the list of infectious diseases that these herds must cope with; a situation that is both unfortunate and preventable. Paratuberculosis control is an added cost to production as the infected herds must invest in routine diagnostics and herd management changes to deal with the problem. Antiquated import testing regulations based on old fashioned tests applied to individual animals (rather than herds of origin) facilitate this mistake. OIE (World Organization for Animal Health) is clearly at fault for failing to promote robust international trade testing protocols thereby allowing paratuberculosis spread to continue, decades after scientists described appropriate biosecurity measures for paratuberculosis; herd-level testing with purchase of herd replacements only from herds with a high probability of being *M. paratuberculosis*-free.

At the herd level, most owners perceive that the cost of the disease, paratuberculosis, is less than the cost of the programs to control it. As a result, only those owners who see obvious clinical disease in too many cows seek veterinary help to control the infection. The majority of commercial herds (herds not selling breeding stock) see no economic motivation to test for paratuberculosis or change management practices to limit its spread.

Cattle breeders fear that a paratuberculosis diagnosis will terminate their ability to sell breeding stock and so they intentionally avoid testing for the infection. Only when they eventually sell an *M. paratuberculosis*-infected animal to another breeder do they discover that their herd is infected. Some of those owners then take this "learning opportunity" to heart, seek veterinary help, and take appropriate actions to rid their herd of the infection; yes, it can be done! Others go into denial, discounting the importance of paratuberculosis, continuing "business as usual", and thereby fostering the spread of paratuberculosis to other breeder herds whose owners are ill-informed about the importance of buying herd replacements only from test-negative herds.

Consequences

The largely uncontained global epizootic of paratuberculosis results in steadily increasing dissemination of *M. paratuberculosis* into the environment. This has consequences for both domestic animals and free-ranging wildlife. The list of wildlife found to be *M. paratuberculosis*-infected is long, and growing. Examples include; North American Bison,

deer, elk, Big Horn Sheep, Rocky Mountain Goats, raccoons and feral cats in the U.S.; fox, stoat, weasel, hare, badgers in Scotland; kangaroos and wallabies in Australia; Guanaco in Chile; and Fallow Deer in Spain. In zoos, paratuberculosis threatens multiple species of hoofed-stock and has also been diagnosed in nonhuman primates. It remains uncertain if *M. paratuberculosis* infections in wildlife are productive infections that threaten wildlife health and pose a risk for spread back to domestic species, or are simply dead-end hosts. For ruminant wildlife, *M. paratuberculosis* is definitely a health problem. Dead-end host or not, this partial list of susceptible animals highlights the capacity of *M. paratuberculosis* to jump species and the expanding impact of this pathogen on the health of all animals.

Humans apparently belong on the list of species susceptible to *M. paratuberculosis* infection. A meta-analysis by Feller et al. (Lancet Infect. Dis. 7:607, 2007) concluded that *M. paratuberculosis* is consistently associated with Crohn's disease. The most recent study by Timms et al. in Australia (PLOS ONE DOI:10.1371, February 5, 2016) is typical of most such studies. They found that 6 of 21 (29%) Crohn's patients were positive for *M. paratuberculosis* infection (PCR positive for IS900, and F57, and a 16s rRNA gene common to all mycobacteria). The organism also was successfully cultured from one of these infected Crohn's patients.

While most in the medical community continue to discount the significance of these findings, it is almost certain that humans are becoming infected and that the most probable means of infection is via the food supply. Infected dairy herds yield *M. paratuberculosis*-contaminated milk. Pasteurization is not 100% effective at killing the organism as evidenced by multiple reports of culturing viable *M. paratuberculosis* from retail dairy products. Cattle, sheep and goats with clinical signs of paratuberculosis are slaughtered and in most all cases used for human consumption. Cooking meat to a well-done condition may effectively kill *M. paratuberculosis* but most foodies would agree that this is not the ideal way to enjoy meat.

Predictions

The 1924 prediction by Larson et al. was right; paratuberculosis has become a more troublesome scourge than tuberculosis (TB), at least in most developed countries. Like bovine TB, paratuberculosis will prove to be a zoonotic disease significantly impacting both animal and human health. Given that all pathogenic mycobacteria are zoonotic, this is not a great leap of logic.

Our strategies for dealing with this panzootic/pandemic will probably not resemble the approaches taken for bovine TB control, however. Countries can no longer afford national control programs like those employed for bovine TB and animal agriculture lacks political power to convince governments to spend the huge sums of money required for such programs. But, this should not paralyze us. Instead, we should find creative ways to foster paratuberculosis control at the level of the animal, herd, region, and internationally. In search of ways to motivate producers, some will say that we need more educational programs. I disagree. We have abundant educational materials in print and on the web and our community of bovine practitioners are well-versed in paratuberculosis and able to teach their producer clients. What we need is a better business plan.

Facing the facts

Producers run businesses that must make a profit if they are to survive. Saddling them with 100% of the cost to control a disease that has health and economic implications for the entire industry, and quite probably food safety and human health, is simply not fair and will never result in real progress. What will motivate producers? The answer is simple: money. We need to put in place economic drivers coupled with industry-driven regulations that make paratuberculosis programs work without creating complex, inefficient and costly governmental programs.

Paratuberculosis can be controlled and quite possibly even eradicated. It is a fecal-oral transmitted disease just like salmonellosis and a host of other infectious diseases. Blocking transmission is simply a matter of hygiene. To insure that hygienic measures are effective it is equally important to find the infectious cows in a herd using diagnostic tests and to cull the test-positives or isolate them away from susceptible calves. None of this is "rocket science". We have herd risk assessment tools to identify necessary herd management changes. We have excellent, commercially available diagnostic tests kits available internationally. We have skilled veterinarians to coach producers in the process and insure that they stay on track. The challenge is not in what to do. Rather, the challenge is in doing those things consistently for a long time, e.g. at least 6 years for most dairy herds.

The clock is ticking and time is running out. A large-scale, multi-national clinical trial is underway that may substantiate what case reports have already shown; treatment of Crohn's disease with a cocktail of three anti-*M. paratuberculosis* antibiotics can cure a high proportion of Crohn's cases. No other therapy available today can make this claim. The ability to cure Crohn's disease using anti-*M. paratuberculosis* antibiotics may be the finding that tips the scale of scientific opinion. Consumers are concerned and Crohn's patients are increasingly aware about the potential health risks of *M. paratuberculosis* (<http://thecrohnsinfection.org/>). In addition to expecting high welfare standards for food-producing animals the public also should reasonably expect that these animals are healthy. And, animals with paratuberculosis are not healthy.

The way forward

- Veterinarians must be educators as well as guardians of animal and public health. Limiting animal and human exposure to *M. paratuberculosis* starts on the farm; the critical control point. Bovine practitioners should make paratuberculosis a part of every herd health plan.
- Dairy processors should incentivize producers to test their herds. For example, a novel program in the UK offered to pay all of the testing costs for dairy herds that were found to be 100% test-negative.
- Dairy processors should pay producers more for milk from test-negative herds; thus enhancing product safety, insuring consumer confidence, and partially compensating herd owners for their efforts to attain and sustain a test-negative herd status.
- Breed organizations should implement requirements that cattle sold by their members come from test-negative herds. By announcing such programs well in advance and phasing rules in slowly, breed organization members can collectively move toward this ideal goal. For example: organizations could first require that cattle sold only originate from test-negative dams, then require that cattle sold come from test-negative dams in herds where 30 randomly selected adults are found test-negative, and eventually mandate that cattle for breeding purposes are only sold by herds with a history of being test-negative over multiple years. Programs can begin using low-cost, low-sensitivity tests like ELISAs and eventually move toward higher cost and higher sensitivity tests like PCR on fecal samples. Naturally, veterinarians are vital for insuring the integrity of the entire process beginning with sample collection. As the Chinese proverb states: A thousand mile journey starts with one step. It's time for breeders to take that step.
- Governmental agencies should provide the infrastructure for these market-driven programs; educating veterinarians, licensing diagnostic tests, certifying laboratories and setting standards for herd classification based on likelihood of paratuberculosis-freedom. This infrastructure is already in place in many countries.
- The collective costs for paratuberculosis control will undoubtedly be passed along to consumers. There is nothing unfair about this. They will also be the ultimate beneficiaries of safer food from healthier animals.

Practical Cow-Side Laboratory Tests For The Cattle Veterinarian

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Point-Of-Care Analyzers

The ready availability of point-of-care analyzers will revolutionize on farm diagnostic testing. These analyzers place patient test results immediately into the veterinarians' hands. Diagnosis is therefore facilitated, and appropriate treatment can be instituted without delay. Point-of-care analyzers have great potential to be a practice builder.

The archetypal point-of-care device is the California mastitis test (CMT). For literally a couple of cents per cow, the CMT test provides a cow-side test that provides a clinically useful estimate of the number of somatic cells in the secretion from each quarter, and therefore the likelihood of subclinical mastitis (Schalm and Noorlander, 1957). This information can be used at dry off to guide decisions related to selective dry cow antimicrobial therapy, or used as a screening test to identify cows for milk culture to identify the predominant mastitis pathogens on the dairy. A number of recent clinical examples from our laboratory will be presented demonstrating how the use of point-of care analyzers or equipment can positively impact bovine veterinary practice. Time will tell whether they impact clinical practice to a similar degree than has the CMT. The information in this presentation is additive to that provided by Professor Rollin at the 2006 World Buiatrics Congress (Rollin, 2006); Professor Rollin and his colleagues at the University of Liège in Belgium have a wonderfully equipped practice vehicle that facilitates the field diagnosis of disease and poor performance in cattle. Additional information on field chemistry analysis has also been published elsewhere (Vap and Weiser, 2007).

Glucose Meters To Identify Dairy Cows With Negative Energy Balance

Measurement of plasma glucose concentration ([gluc]) is clinically valuable in periparturient dairy cattle, particularly during the period from day 3 to day 28 of lactation. Plasma non-esterified fatty acid concentration provides the best index of energy balance in lactating dairy cows from 1 to 10 weeks postpartum (Reist et al., 2002) but its current cost (US\$9.00/test) and lack of a suitable point-of-care device prohibits routine use as a monitoring tool. Plasma [gluc] is negatively associated with energy balance in lactating dairy cows to a similar degree as serum [BHB] (Reist et al., 2002); plasma [BHB] also has a marked diurnal change based on time of feeding, whereas plasma [gluc] does not exhibit a diurnal effect (Nielsen et al., 2003). Moreover, plasma/serum [gluc] is negatively associated with plasma/serum [BHB] in dairy cattle from 2 months before to 2 months after calving (Nielsen et al., 2003; Kauppinen, 1983). Blood [gluc] can be measured for approximately US\$0.30/test whereas measurement of blood or plasma [BHB] costs US\$1.30-3.10/test; consequently, four to ten times as many cattle can have their blood [gluc] measured than their blood [BHB] for the same overall cost. This cost differential facilitates the implementation of blood [gluc] as a potentially useful index of energy balance in periparturient cattle.

The Precision Xtra® meter is a low cost electrochemical point-of-care unit for measuring blood glucose concentration ([gluc]) in cattle blood. The meter uses specialized electrochemical test strips that are screen-printed to monitor glucose and β-OH butyrate concentrations in whole blood. The first layer of the test strip contains mesh and surfactants that facilitate retention of erythrocytes and passage of plasma by capillary action to an underlying layer that employs an enzymatic reaction, with the reaction products being detected by generation of an electrical current. The meter uses an algorithm optimized for human blood that

assumes the intra-erythrocyte [gluc] equals the plasma [gluc], and that the hematocrit is similar in humans and cattle. We recently published a study with the primary objective being to determine the accuracy of the meter for measuring plasma [gluc] in dairy cattle (Megahed et al., 2015). Secondary objectives were to characterize the influence of hematocrit and sample temperature on the measured value for [gluc]. Blood and plasma samples (1,109) were obtained from 106 periparturient Holstein-Friesian cattle. Deming regression and Bland-Altman plots were used to determine the accuracy of the meter against the reference method (plasma hexokinase assay). Multivariable regression and linear regression were used to determine the effect of hematocrit and sample temperature on the plasma [gluc] measured by the meter. We found that sample temperature had a significant linear effect on plasma [gluc] as measured by the meter when the reference method [gluc] > 8.8 mmol/L. Intra-erythrocyte [gluc] was 18% of plasma [gluc] on a molar basis. We concluded that the meter utilizes an algorithm that is optimized for human blood and is inaccurate when applied to bovine blood. Until a cattle-specific algorithm is developed, we recommend using plasma as the analyte instead of blood and calculating plasma [gluc] in mg/dL using the equation: $[gluc] = 0.66 \times [gluc]_{p-meter} + 15$, where $[gluc]_{p-meter}$ is the value reported by the meter in mg/dL. If blood is measured, then we recommend using the equation: $[gluc] = 0.90 \times [gluc]_{b-meter} + 15$ where $[gluc]_{b-meter}$ is the value reported by the meter in mg/dL (Megahed et al., 2015).

B-Hydroxybutyrate Meters To Identify Dairy Cows With Hyperketonemia

Hyperketonemia is a common metabolic disorder of dairy cattle in early lactation that is characterized by increased concentrations of β-hydroxybutyrate, acetoacetate, and acetone in blood, plasma, serum, milk, and urine. Measurement of plasma or serum BHB concentration ([BHB]) is considered the gold standard test for diagnosing hyperketonemia in dairy cattle because BHB is the predominant and most stable of the three ketone bodies (Tyopponen and Kauppinen, 1980). The most commonly used cut points for diagnosing hyperketonemia in dairy cattle are plasma or serum [BHB] > 1.0-1.4 mmol/L.

The Precision Xtra® meter is a low cost electrochemical point-of-care unit for measuring blood [BHB] in cattle. Fundamental assumptions of this methodologic approach are that the measured value for [BHB] is minimally affected by changes in hematocrit and the sample temperature. Changes in hematocrit would influence the measured value for [BHB] if the intra-erythrocyte [BHB] differed from plasma [BHB], and it has been known for more than 70 years that intra-erythrocyte [BHB] is approximately one third to one half the value of plasma [BHB] in human and sheep blood (Reid, 1962). These findings strongly suggest that changes in hematocrit are likely to alter the measured value for [BHB] in cattle blood. Measurement of [BHB] in cattle blood samples at < 32°C resulted in lower measured values for [BHB] than those obtained at 37°C (Iwersen et al., 2013). The potential effect of sample temperature had not been explored in method comparison studies, where blood samples for testing are usually obtained from the finger, ear, or foot pad or by venipuncture and immediately analyzed, and consequently are assumed to be at approximately 37.0 to 38.5°C. Because the meter uses an algorithm optimized for human blood that assumes the intra-erythrocyte and plasma [BHB] are equal, and that the hematocrit is similar in humans and cattle, we were interested in characterizing the analytical performance of the meter for the measurement of blood and plasma [BHB] in dairy cattle. Secondary objectives were to characterize the influence of hematocrit and sample temperature on the plasma [BHB] measured by the meter. Accordingly, blood and plasma samples were obtained from 106 periparturient Holstein-Friesian cattle and 15 lactating Holstein-Friesian cows with experimentally-induced electrolyte and acid-base imbalances. Meter performance was evaluated using Deming regression and Bland-Altman plots. Multivariable and linear regression analysis was used to determine the effect of hematocrit and

sample temperature on the plasma [BHB]. We found that the meter was linear up to a [BHB] of 3 mmol/L as measured by the reference method. An increase in hematocrit increased the measured value for [BHB]; this result was partially explained by intra-erythrocyte [BHB] being much lower than plasma [BHB]. Changes in sample temperature caused a linear change in [BHB]meter-plasma > 3.0 mmol/L. We concluded that the meter algorithm that calculates plasma [BHB] in units of mmol/L is not accurate when applied to bovine blood. Using plasma at 37°C as the analyte instead of blood and application of the equation $[BHB]=0.54 \times [BHB]_{\text{meter-plasma}} + 0.17$ increases the accuracy of the meter. If blood is measured, we recommend using the equation: $[BHB]=0.62 \times [BHB]_{\text{meter-blood}} + 0.20$.

Portable Ion-Selective Electrode Meters For Determining Whole Blood, Plasma, Urine, Milk, and Abomasal Fluid Potassium Concentrations In Dairy Cattle

Hypokalemia is common in lactating dairy cattle with abomasal displacement or volvulus, developing in response to alkalemia secondary to sequestration of chloride in the gastrointestinal tract (Constable et al., 1991). Low feed intake, obligatory loss of potassium in milk (1.4 g of K/L of milk), hyperglycemia, and hypovolemia are also considered main predisposing factors (Constable et al., 2013). Hypokalemia is also common in lactating dairy cattle with abomasal impaction (Wittek et al., 2005) and clinical mastitis (Smith et al., 2001). Lactating dairy cattle with hepatic lipidosis and cattle receiving multiple injections of isoflupredone acetate as part of the treatment of ketosis are more susceptible to hypokalemia (Sattler and Fecteau, 2014). Development of optimal treatment protocols for hypokalemic dairy cattle requires accurate, rapid and repeated measurements of whole blood, plasma, or serum potassium concentration ($[K^+]$) because the clinical signs of hypokalemia are nonspecific and excessive potassium administration can be fatal. Two low cost ion-selective electrode (ISE) hand-held meters (CARDY C-131, LAQUAtwin B-731; Horiba, US) have recently become available for measuring the potassium concentration ($[K^+]$) in biological fluids. We were interested in characterizing the analytical performance of the ISE meters in measuring $[K^+]$ in bovine whole blood, plasma, urine, milk, and abomasal fluid. We completed 6 method comparison studies using 369 whole blood and plasma samples from 106 healthy periparturient Holstein-Friesian cows, 138 plasma samples from 27 periparturient Holstein-Friesian cows, 92 milk samples and 204 urine samples from 16 lactating Holstein-Friesian cows, and 94 abomasal fluid samples from 6 male Holstein-Friesian calves. Deming regression and Bland-Altman plots were used to characterize meter performance against reference methods. The CARDY ISE meter applied directly in plasma measured $[K^+]$ 7.3% lower than the indirect ISE reference method, consistent with the recommended adjustment of +7.5% when indirect ISE methods are used to analyze plasma. The LAQUAtwin ISE meter run in direct mode measured fat free milk $[K^+]$ 3.6% lower than the indirect ISE reference method, consistent with a herd milk protein percentage of 3.4%. The LAQUAtwin ISE meter accurately measured abomasal fluid $[K^+]$ compared to the indirect ISE reference method. The LAQUAtwin ISE meter accurately measured urine $[K^+]$ compared to the indirect ISE reference method, but the median measured value for urine $[K^+]$ was 83% of the true value measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES). We concluded that the Horiba and LAQUAtwin ISE meters provide practical, low cost, rapid, accurate point-of-care instruments suitable for measuring $[K^+]$ in whole blood, plasma, milk, and abomasal fluid samples from cattle. Ion selective electrode methodology is not suitable for measuring $[K^+]$ in bovine urine.

Measuring Maternal Pelvic Area And Fetal Hoof Circumference At Parturition To Predict Dystocia

Dystocia remains a major problem in the dairy industry as it increases cow and calf mortality, decreases milk yield, delays uterine involution, and decreases reproductive performance, ultimately resulting in

substantial financial loss. Fetopelvic disproportion is the most common cause of dystocia in dairy cattle (Mee, 2008). Accurate prediction of dystocia allows early and appropriate intervention, thereby decreasing morbidity and mortality of the dam and fetus, improving animal welfare, and reducing economic losses.

We hypothesized that the measurement of calf front hoof circumference, maternal intrapelvic and selected morphologic dimensions in late gestation, and an estimate of the dam's body weight would be predictive of calving difficulty score in dairy cattle as previously documented for some of these factors in primiparous beef cattle (Short et al., 1979). We also hypothesized that the ratio of calf front hoof circumference to maternal intrapelvic area or width has clinical utility to predict dystocia in dairy cattle, as these ratios directly reflect the magnitude of fetopelvic disproportion. We investigated our hypotheses using a convenience sample of 103 late gestation Holstein-Friesian heifers and cows (Hiew et al., 2016). Intrapelvic height and width of the dam were measured using a pelvimeter and the intrapelvic area was calculated. Calf front hoof circumference and birth weight were also measured. Data were analyzed using Spearman's correlation coefficient (r_s), Mann-Whitney U test, and binary or ordered logistic regression; $P < 0.05$ was significant. Calving difficulty score (1 to 5) was greater in heifers (median, 3.0) than cows (median, 1.0). Median intrapelvic area immediately before parturition was smaller in heifers (268 cm²) than cows (332 cm²), whereas front hoof circumference and birth weight of the calf were similar in both groups. Calving difficulty score was positively associated with calf birth weight in primiparous cattle ($r_s = 0.39$) and cows ($r_s = 0.24$). Binary logistic regression using both dam and calf data indicated that the ratio of front hoof circumference of the calf to the maternal intrapelvic area provided the best predictor of dystocia (calving difficulty score = 4 or 5), with Se = 0.50 and Sp = 0.93 at the optimal cutpoint for the ratio (> 0.068 cm/cm²). We concluded that determining the ratio of calf front hoof circumference to maternal intrapelvic area has clinical utility in predicting calving difficulty score in Holstein-Friesian cattle (Hiew et al., 2016).

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An Exciting Future For The Education Of Food Supply Veterinarians

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We are facing a period of rapid change in Food Animal Practice. Student interest in bovine practice has declined, associated with an increase in wealth and prosperity in developed countries, an emphasis on lifestyle, and the rise in societal and economic importance of companion animals (Baumgartner, 2002; Radostits, 2003). At the same time, the development of large scale commercial cattle enterprises and globalization of agriculture have increased the demand for advanced knowledge and skills, as well as increasing societal concerns about food security and ecosystem health (Eyre et al., 2004). These changes dictate alterations in the educational process of future bovine practitioners.

The Evolution Of Bovine Veterinary Practice

There are two main models of agricultural production, the family farm and agribusiness. In simplified terms, the species is the unit of production in the family farm model and animals are frequently identified by name. In contrast, the farm is the unit of production in the agribusiness model and animals are identified by number.

The Family Farm model

The family farm model, which dominated agricultural production until the latter half of the 20th Century, consisted of numerous small diversified units of production. The nature of veterinary services provided to this community are primarily individual and emergency; the lack of high density populations meant that it was difficult to identify economically important and production limiting diseases. The diversification present on each farm also meant that the producer often lacked rudimentary health and disease knowledge. The producer therefore depended heavily on the veterinarian to provide treatment and routine husbandry procedures such as dehorning and castration. Accordingly, veterinarians and the veterinary curriculum focused on the individual "diseased" animal in the tradition of James Herriot. In the traditional family farm model, producers frequently did not participate in the treatment of sick cattle or the implementation of preventative programs, and most veterinarians maintained a time consuming individual mixed animal practice that returned a moderate income. Veterinarians also had more influence on drug use and treatment decisions.

The veterinary curriculum addressed the needs of mixed animal practitioners by providing a broad based education that was discipline based, because this was the most efficient way of transferring general veterinary medicine principles to all domestic animals. The information explosion and impressive technological advances in human medicine over the last 50 years drove large scale specialization in human medicine, with the development of numerous discipline based specialty boards. The veterinary profession in North America followed the lead of the medical profession and in the late 1960's started a system of specialization that was discipline based (Fig. 1). The demand for clinically based veterinary specialties, such as Surgery and Internal Medicine, grew as households had more disposable income and people become increasingly interested in maintaining the health and prolonging the life of their companion animals.

The Agribusiness Model

Economic pressures, sociopolitical changes, and technological advances caused a shift from the family farm to the agribusiness enterprise in

the second half of the 20th century. In essence, small scale diversified units of production were transformed into larger scale, focused units of production. Accompanying this change has been the development of four main cattle industries, dairy, beef (cow-calf), feedlot, and veal, with additional subspecialization into other areas such as contract heifer raising, bulls, artificial breeding, embryo transfer, and cloning.

The presence of high density populations meant that was easier to identify economically important production limiting cattle diseases, and to economically compare treatment and control methods. Agribusiness specialization also meant that the producer or farm manager possessed detailed health and disease knowledge relevant to their enterprise, and were less reliant on the veterinarian to provide or recommend routine treatment and husbandry procedures. In the agribusiness model, farm personnel frequently do all the routine treatment of sick cattle and implement preventative programs, with veterinarians assuming more of a consultant and trouble-shooting role (Guterbock, 2001). This has led to the widespread development of large group practices in North America, Australia, and New Zealand, with attendant subspecialization along discipline lines. The veterinary curriculum has addressed the changed landscape by adding courses on Production Medicine, Epidemiology, and more recently, Food Security.

Education Of Future Bovine Practitioners In The Professional Program

The last twenty years has seen greater worldwide interest in evaluating the effectiveness and efficiency of the educational process. The United States Department of Education has charged the American Veterinary Medical Association (AVMA) Council on Education (COE) to oversee the accreditation process for Colleges/Schools of Veterinary Medicine in the United States. The 2016 version of the COE Accreditation Policies and Procedures lists the requirements of an Accredited College of Veterinary Medicine. Section 7.11 (Standard 11) of the Accreditation Policies and Procedures (American Veterinary Medical Association, 2016) deals with outcomes assessment and states the following: "New graduates must have the basic scientific knowledge, skills, and values to provide entry-level health care, independently, at the time of graduation." The fundamental goal of defining entry-level competencies has been addressed in many countries under similar terms such as "Day one" or "Entry level" or "Year one" competencies and "Practice ready veterinarians". The World Organization for Animal Health (OIE, [Office International des Épizooties]) developed guidelines for a model core veterinary curriculum in 2012 that are offered primarily to developing and in-transition countries as an initial step to enhance and sustain national Veterinary Services (World Organization for Animal Health, 2012).

Section 7.11 of the AVMA COE Accreditation Policies and Procedures states: "The college must have processes in place whereby students are observed and assessed, with timely documentation to assure accuracy of the assessment for having attained each of the following competencies:

- 1. comprehensive patient diagnosis (problem solving skills), appropriate use of diagnostic testing, and record management
- 2. comprehensive treatment planning including patient referral when indicated
- 3. anesthesia and pain management, patient welfare
- 4. basic surgery skills and case management
- 5. basic medicine skills and case management
- 6. emergency and intensive care case management
- 7. understanding of health promotion and biosecurity, prevention and control of disease including zoonoses and principles of food safety
- 8. client communications and ethical conduct
- 9. critical analysis of new information and research findings relevant to veterinary medicine."

Defining entry level medicine, surgery, and anesthesia skills for food animal practice

The successful veterinarian needs to achieve a minimum standard in knowledge, skills, and attributes. A critical issue is defining the relevant day one medicine, surgery, and anesthesia skills for food animal practice, and developing efficient and effective methods for developing and documenting competency in these skills. Surprisingly, this fundamental requirement of defining skills has been rarely undertaken. It is vital that each country or region define the basic medicine, surgery, and anesthesia knowledge and skills related to food animal practice. Entry level knowledge, skills, and values need to be identified because most veterinary students enter clinical practice, there is limited time and resources available for lectures and laboratories in the curriculum, bovine surgery and anesthesia laboratories are expensive requiring an adequate return on investment, and societal concerns regarding the use of live animals versus non-animal alternatives. The skills should be periodically re-evaluated because of the evolving role of the bovine practitioner in response to changes in the needs of industry (fewer, larger herds, farm laborers performing some veterinary procedures), society (increased interest in animal welfare, food safety, locally sourced products), and veterinary practice (perceived change in emphasis from individual animal diagnosis and treatment to herd health and production, with post graduate sub-specialization).

The University of Illinois at Urbana-Champaign has been a pioneer in veterinary curriculum development. We investigated the basic medicine, surgery, and anesthesia skills related to food animal practice in 1998-1999 by conducting an anonymous mail survey of US veterinarians in bovine practice (Morin et al., 2002a, 2002b). To our knowledge, this was the first such survey of bovine veterinarians conducted world-wide. The response rate of 60% with 1,450 respondents was outstanding for a mail survey and clearly indicated the interest in practicing veterinarians in ensuring that colleges of veterinary medicine were producing graduates with required entry level competencies. The questionnaire asked respondents to rate the frequency and proficiency of 148 veterinary skills, including 43 surgical skills, 10 anesthesia/restraint skills, 42 individual animal diagnosis/medicine skills, 42 production medicine/herd health skills, and 11 reproduction skills. The questionnaire used 5 point Likert ratings for frequency of 0 = never, 1 = rarely (< once/month), 2 = occasionally (1-2x/month), 3 = frequently (once/week), 4 = all the time (> once/week). Frequency scores for all 148 procedures and skills ranged from 3.4 ± 0.9 to 0.1 ± 0.3; 40/148 (27%) procedures/skills and 11/53 (20%) surgical and anesthetic procedures/skills had scores > 2.0 (performed > 1x/month). These procedures and skills occurred commonly enough that they should be included in the core curriculum (didactic and laboratory). The top 10 most frequently used medicine and animal production procedures and skills (frequency score in parentheses) were injections (3.7), oral medication (3.5), pregnancy diagnosis (3.4), venipuncture (3.4), treatment of pneumonia (3.2), management of dystocia (3.0), treatment of diarrhea (3.0), auscultation of lungs (3.0), IV fluid therapy (3.0) and cardiac auscultation (2.9). Other medicine and animal production procedures and skills of interest were auscultation of the gastrointestinal tract (2.9), development of a vaccination program (2.7), development of an anthelmintic program (2.6), treatment of metritis (2.5), treatment of mastitis (2.3), rumenocentesis (1.0), ration formulation (0.9), transtracheal aspiration (0.6), cerebrospinal fluid collection (0.3), and electrocardiography (0.2) (Morin et al., 2002a).

For comparison, the top 10 most frequently used surgical and anesthetic skills were castration (3.0), epidural anesthesia (2.8), dehorning (2.7), IV or IM sedation (2.6), tattooing (2.6), treatment of foot lesions (2.5), wound management (2.4), ear implanting (2.4), flank anesthesia (2.3), and vaginal prolapse repair (2.2). The least frequently used surgical and anesthetic skills/procedures were fracture casting (1.2), joint lavage (0.7), use of surgical hyperthermia (0.7), rectovaginal fistula repair (0.7), umbilical resection (0.7), use of cryonecrosis (0.6), liver biopsy (0.4), tracheotomy/tracheostomy (0.4), tracheal intubation (0.4) and

inhalation anesthesia (0.3) (Morin et al., 2002b). As expected, there was an effect of practice type on the most common knowledge and skills. Dairy practitioners expected higher proficiency in laparotomy, surgical correction of left displaced abomasum, teat surgery, and rectovaginal fistula repair. Cow-calf (beef) practitioners expected higher proficiency in urethrotomy, urethrostomy, roping, and branding.

Similar findings to our paper were reported in a 2004 paper from the University of Missouri (Miller et al., 2004). In a 2006 paper focusing in defining the required level of competency for reproductive skills at graduation (competency score of 1 = not at all competent; competency score of 5 = extremely competent), the highest competency scores were transrectal palpation for evaluating reproductive status (3.5), dystocia management (3.4), breeding soundness exam – males (3.4), herd health management (3.3), treatment of reproductive tract disease (3.3), cesarian section (3.1), and treatment of infertility (3.0) (Kustritz et al., 2006). These findings were reinforced in a 2013 paper summarizing the skills required for food animal practice in western Canada (Luby et al., 2013). Individual animal medicine and surgery skills were performed more commonly than herd health skills. The most important skills identified were those required for physical examination, treatment of common disorders, general surgery, and basic theriogenology.

These surveys indicate that physical examination and technical skills continue to be valued more highly for entry level veterinarians than evaluative, integrative, and problem solving skills. This is a disappointing finding because it suggests that an apprenticeship model (vocational school training) may provide a more appropriate educational program for bovine practice rather than a professional program (University degree). However, bovine practice is a profession and not a trade, because in the future evaluative, integrative, and problem solving skills will be required for bovine practitioners to adequately serve and advance cattle industries.

A suitable curriculum for the future bovine practitioner

The components of the required skill set for bovine practice have been described elsewhere (Trout and Osburn, 1989; Baumgartner, 2002; Hird et al., 2002; Radostits, 2003; Kyriakis et al., 2004), but include the traditional individual animal curriculum as well as knowledge and mastery of the following components:

- Animal production systems, including agricultural economics, agricultural engineering and facility design, and globalization
- Disease control through strategic management, treatment, metaphylaxis, and vaccination
- Food security, biosecurity, and bioterrorism
- Nutrition for high production
- Population health, including evaluation using clinical epidemiology
- Public health, zoonotic disease, ecosystem health, and waste management
- Research methodology, including good clinical practice, good laboratory practice, study design, and biostatistics
- Risk assessment and risk management, including application of hazard analysis and critical control points

Additional skills needed relate to “nontechnical” or “soft” competencies, including professional characteristics related to core values and motivation, as well as suitable personality traits for supervising and motivating staff and engaging with the public (Walsh et al., 2001). Bovine practitioners will also need the skills to adapt to changing industries (Luby et al., 2013) and technologies.

Some of these modules need to be part of the veterinary curriculum for the bovine practitioner, whereas others are better situated as post graduate modules in certificate programs. It is important to recognize that the veterinary degree is not the end of the educational process, and that society demands veterinarians to be life-long learners. Implementation

of some of these modules into the veterinary curriculum will require contraction of the core curriculum and expansion of the elective curriculum (Blackwell, 2001; Eyre, 2002; Nielsen, 2003; Radostits, 2003). Whether implementation of these modules into the veterinary curriculum necessitates a move towards designated or limited licensure remains to be determined.

Students should be exposed to food animal cases in both a hospital and ambulatory setting; use of only one of these experiential programs is insufficient for day one competency in bovine practices. Although concerns regarding biosecurity at Veterinary Teaching Hospitals related to infectious disease, including Infectious Bovine Rhinotracheitis, Salmonella, Foot and Mouth Disease, and virulent strains of *Staphylococcus aureus* mastitis and *Mycoplasma bovis* mastitis are valid, experience has shown that biosecurity can be satisfactorily addressed related to cattle admissions. There is also no doubt that Veterinary Teaching Hospitals are strong and the primary vehicle for clinical education at leading Colleges of Veterinary Medicine. Ideally, the surrounding region should have a high livestock density for food and fiber animals, but this is not always possible. Having trucks that drive further afield to bring cases to and from the hospital has worked well for the Veterinary Schools in Hannover and Berlin in Germany and this approach merits wider adoption. Teaching hospitals permit more students to see great learning cases under optimal handling conditions, and provide more time for students to be involved in case management, than ambulatory or off-site experiences where time constraints are real and handling facilities may be substandard. More importantly, teaching hospitals provide veterinary students to understand disease evolution and the response to treatment, such as changes in appetite and hydration status, fecal characteristics, respiratory rate, demeanor that is not available in cross-sectional experiences in ambulatory, where animals under treatment are usually not examined each day. Hospital activities should be well integrated with discovery and engagement activity in the clinical department and relevant to the cattle industries in the region.

The main weakness with a hospital based caseload is the failure to see the animal in its environment, where the animal is part of a population. Future bovine veterinarians need to develop skills in evaluating the individual animal in the context of the population; for instance, the impact of poor building design and ventilation, poor feed quality, and inadequate nutrition on disease prevalence and severity. It is therefore important to complement the in house hospital experience with on farm experience, which could be attained using an ambulatory service, partnership with a busy food animal practice with embedded university clinicians, such as the Dairy program at the University of Melbourne in Australia (University of Melbourne Dairy program, 2016), or providing veterinary service to a large dairy farm with > 2,000 cows.

Postgraduate Education For Bovine Practitioners

Universities and Colleges of Veterinary Medicine must improve the depth and breadth of postgraduate education opportunities for veterinarians, primarily by offering learning modules and certificate programs as continuing education opportunities for veterinarians. These certificate programs could be on-site, a mixture of scheduled on-site and asynchronous distance learning programs, or entirely on-line, depending on the knowledge and skills in each module. The certificate programs would also meet postgraduate continuing education requirements in order to maintain a license to practice. Massive open online courses (MOOC's) offer one vehicle for on-line learning. The University of Illinois has been an early adopter of on-line education and a MOOC delivered in 2015 titled "Sustainable Food Production Through Livestock Health Management" provided a 6 week course of study comprising 7-9 hours/week relevant to bovine practitioners (Coursera, 2015). Students could take the course for free or receive a certificate for a fee from Coursera® indicating successful completion of the course and an acceptable result on the accompanying quizzes.

Another example of certificate programs for food animal practitioners is the Executive Veterinary Program (EVP) at the University of Illinois. Since 1991 the University of Illinois College of Veterinary Medicine has offered the EVP certificate in swine health management (University of Illinois College of Veterinary Medicine, 2016.). The program was developed to enable veterinarians to experience personal and professional development in the areas of industry trends, communication, leadership, economics and finance, marketing, legal and ethical issues, public health concerns and strategic planning. The EVP programs have been popular as they fit into their busy schedules of veterinarians being scheduled over weekends during a 12 month period, while optimizing engagement with industry leaders and providing substantial networking opportunities.

Human medicine has proceeded firmly along the path of post graduate internship and residencies, which effectively restrict the practice of medicine to specific subdisciplines. Societal needs regarding access to appropriately priced and competent veterinary services will not support the mandated requirement of internships and residencies in veterinary medicine in the absence of state provided insurance programs (such as in human medicine). Moves to lengthen the veterinary curriculum by mandating a required low paid internship year should be vigorously resisted. A more appropriate approach is to let the market place set the salary for the first year graduate based on their skill set and talents similar to Law practice; bovine practitioners that have completed modules relevant to cattle industries will command a higher starting salary than other graduates that do not have relevant focused work experiences.

There is an important role for veterinarians with graduate degrees (such as M.S., M.P.H, M.B.A, or Ph.D) in government and industry positions. There is also a role for veterinarians with specialist degrees in bovine practice, with important caveats. The discipline based system of specialization (Fig. 1) has worked well for companion animals, but has failed miserably when applied to agricultural animals. The principle reason for this failure is that most specialty boards are focused on attaining rather than applying a set of skills; agribusiness correctly places an individual animal and associated techniques as just one part of an integrated enterprise. A veterinary specialist working in the cattle industry must consider economics, practicality, animal welfare, population health, food security, ecosystem health and many other factors in every decision or recommendation.

The failure of the discipline based system to deliver an appropriately trained specialist to the cattle industry has been recognized in North America for some years. Practitioner specialty boards, which are species/industry based, were introduced in 1978. In 1997 the Surgery specialty board split into Small Animal and Large Animal components, and in 2002 the Large Animal component further split into Equine only or mixed large animal (80% Equine, 20% Food producing animals). In 2002 the Internal Medicine specialty split into Small Animal and Large Animal components and it is likely that the Large Animal Internal Medicine specialty will split into Equine and Food producing sections. These changes represent temporary patches for a failing system. Europe and Australia were late to adopt veterinary specialization and saw the failure of the discipline based approach in the US. Accordingly, European specialization can be organ, species, or discipline oriented (Lumeij and Hertage, 2006).

Approximately half of the Colleges of Veterinary Medicine in North America align their in house clinical service units along discipline lines (such as Large Animal Medicine, Large Animal Surgery), whereas the remainder organize their in house clinical service units on species lines (such as Ruminant Medicine and Surgery, Equine Medicine and Surgery). A discipline based approach provides an inappropriate organizational structure for hospital based activities many reasons. First, clients do not request the services of Large Animal Internist, Surgeon, or Reproductive specialist, instead they request the services of a Ruminant Internist, Equine Surgeon, or Bovine Theriogenologist, depending upon their animal and the nature of the problem. Large animal clients therefore identify first with the species and industry, and then with the type of specialty. Second, the only employment opportunities for a

Large Animal specialist are located in 14 North American Colleges of Veterinary Medicine; positions for Large Animal Internists, Surgeons, or Theriogenologists or other specialists simply do not exist in private practice (although many positions exist for species-focused specialists). Third, the annual in house case is statistically and significantly lower in large hospitals organized in a discipline, rather than species, manner (Tyler et al., 2002). Finally, most large animal veterinary practitioners define themselves as multiple species (mixed) practitioners, or predominantly dairy, beef, equine, swine, or poultry practitioners. Because we should be training our professional students for a job in the real world, the organizational structure of in house activities in Clinical Science departments should reflect the real world situation and be organized along species/industry lines.

Fields of specialization should therefore first and foremost be species/industry based; for cattle industries this means that practitioners will first specialize in ruminants (or cattle), with further subspecialization in dairy, beef (cow-calf), feedlot, and veal. Under the species/industry umbrella, there is ample scope for veterinary subspecialization in a discipline-based specialty, such as Reproduction (Theriogenology), Internal Medicine, Nutrition, Food Quality and Security, Epidemiology, and Surgery (Fig. 2). This model has been adopted in Europe with the formation of the European College of Bovine Health Management in 2003 (Lumeij and Herrtage, 2006). The main objective of the College of Bovine Health Management is to advance the health and production of cattle in Europe by providing expert care for cattle; encouraging research and other contributions to the science and practice of bovine herd health management including; animal husbandry, internal medicine, surgery, obstetrics and reproductive management, as they relate to the epidemiology, pathogenesis, diagnosis, therapy, prevention, and control of diseases directly or indirectly affecting cattle.

Conclusions

Bovine practitioners must obtain skills and knowledge to adequately service cattle industries and thereby meet societal needs. This requires contraction of the core curriculum and expansion of the elective curriculum. The administrative structure of Colleges of Veterinary Medicine and particularly the Clinical Sciences departments should be organized along species/industry lines rather than discipline lines. Postgraduate specialization should also be organized along species/industry lines rather than discipline lines, with increased availability of modules or certificate programs related to areas relevant to cattle industries. Discipline based specialties (such as Internal Medicine, Surgery, Theriogenology) will become increasingly irrelevant to bovine practice unless they are placed in the context of one or more cattle industries.

References

For tables and references, please go to the following webpage:
<http://imgpublic.mci-group.com/ie/PCO/PeterConstableReferences.pdf>

A lesion oriented, life cycle approach to preventing lameness in dairy herds

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Introduction

We have a global lameness crisis in our dairy industry. The worldwide prevalence of lameness in dairy herds (defined as a cow walking with noticeable weight transfer and a 'limp') is approximately 26% across studies based in Austria, Canada, China, Finland, Germany, Italy, Netherlands, New Zealand, Norway, UK and the US (e.g., Amory et al., 2006; Barker et al., 2010; Chapinal et al., 2014; Cook, 2003; Cook et al., 2016; Dippel et al., 2009; Fabian et al., 2014; Kielland et al., 2009; Popescu et al., 2014; Sarjokari et al., 2013; von Keyserlingk et al., 2012), with a trend toward lower prevalence in grazing or mixed housing and grazing systems (e.g. 16.5% in Amory et al., 2006; 8.3% in Fabian et al., 2014; and 15% in Haskell et al., 2006), and a higher prevalence in confinement housed freestall herds (e.g. 31% in Chapinal et al., 2014; 54.8% in North East US dairy herds in von Keyserlingk et al., 2013).

Despite research and a significant improvement in our understanding of the causes of lameness over the last three decades, we appear to be fighting a losing battle, and the problem has been associated with increasing intensification of the dairy industry, higher milk production and confinement housing, with the obvious conclusion that lameness is an inevitable consequence of these decisions. Consumers carry an expectation that cows should graze and appear to place considerable value on cattle having access to the outdoors, where they have fresh air and freedom to roam (Cardoso et al., 2016). They emphasize the need for humane care of the animals (Cardoso et al., 2016), so the sustainability of the industry is threatened when the general public learns that production systems do not meet their expectations – and lameness is an obvious problem that has been and should continue to be a high priority for us to resolve.

What Causes Lameness in Dairy Cattle?

The aetiopathogenesis of a variety of hoof lesions has been researched and reviewed extensively (eg. Bicalho and Oikonomou, 2013; Cook and Nordlund, 2009; Cook, 2015; Evans et al., 2015), centering on genetic, nutritional, hormonal, mechanical, infectious and environmental factors.

Across numerous surveys in different production systems, three lesions emerge consistently as the most significant contributors to lameness – digital dermatitis (DD), white line disease (WLD) and sole ulcer (SU) (eg. Barker et al., 2009; Defrain et al., 2013). Our ability to impact lameness globally will depend on developing effective control strategies targeted at these three lesions. I will concede that some differences do exist between production systems and some countries. For example, DD has yet to become a dominant hoof lesion in New Zealand and Australia, likely due to the absence of environmental risk factors. However, the disease has spread in association with confinement housed dairy systems around the world, and now even in these locations DD is appearing at a low prevalence (Chesterton, 2015). In grazing systems, WLD appears to dominate, with sole bruising and axial wall fissures often reported. It is however important to note that a healthy sole is unlikely to 'bruise' unless the sole thickness is compromised, suggesting this as an underlying cause. We know thin soles emerge as a significant problem in larger confinement dairy systems in association with toe ulceration (Shearer et al., 2006), where cows are asked to walk long distances to and from the parlor for milking. It is therefore likely that hoof wear is the underlying issue in both, due to exposure to the track (grazing herds) or concrete alley (confinement herds).

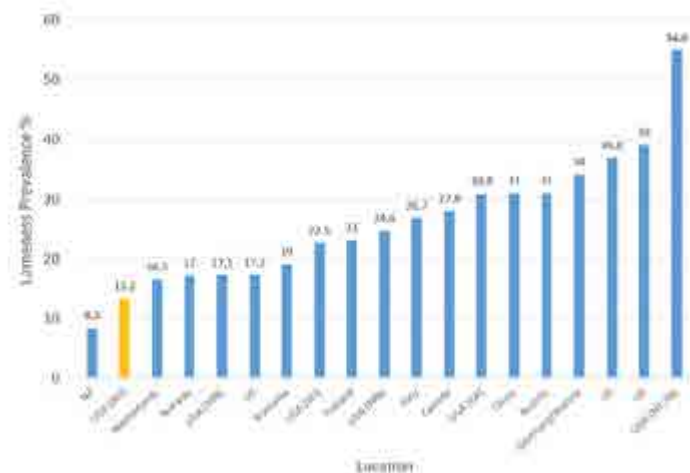


Figure 1. Worldwide prevalence of lameness in dairy herds by location from the peer reviewed literature since 2003

I will contend that we now know more than ever what causes lameness, and while we still have more to learn, we know enough currently to solve the global lameness problem in our dairy industry.

A Life Cycle Approach

No matter what the causation of lameness, once the cow develops a lesion, they are at much greater risk for developing the same lesion again in the next lactation (Oikonomou et al., 2013), likely due to permanent anatomical changes to the structure and function of the claw – including the fat pad, the suspensory apparatus and the pedal bone itself (Table 1).

Lesion	Parity	Lesion Status	Lactation Adjusted Incidence			P-value
			2	3	≥4	
WLD	1	0	6	11	15	<0.01
		1	20	21	24	
	2	0		9	13	<0.01
		1		20	18	
	3	0			10	<0.001
		1			21	
SU	1	0	12	20	26	<0.001
		1	44	32	23	
	2	0		15	24	<0.001
		1		40	30	
	3	0			18	<0.001
		1			41	
DD	1	0	7	7	8	<0.001
		1	32	15	10	
	2	0		5	7	<0.001
		1		19	12	
	3	0			5	<0.001
		1			14	

Table 1. Lactation adjusted incidence of lameness lesion (white line disease = WLD, sole ulcer = SU and digital dermatitis = DD) by lesion status (0 = no lesion, 1 = lesion) in the previous parity (1-3). (from Oikonomou et al., 2013)

We are also aware that while claw horn disease is relatively uncommon in heifers, DD infection may impact 20-30% of heifers after breeding age in many rearing facilities, likely as a result of the same poor leg hygiene risk factors that have exacerbated the problem in mature cows. Laven and Logue (2007) and Holzhauer et al. (2012) have demonstrated the importance of the pre-partum period in affecting DD occurrence during the following lactation, and Gomez et al. (2015) were able to demonstrate increased risk for DD in primiparous cows when they suffer one or more episodes of DD during the rearing period, compared to heifers that are unaffected during the rearing period.

DD affects younger cows, with incidence peaking typically in the 1st or 2nd parity, while SU and WLD incidence increases with age to around the 4th lactation (Oikonomou et al., 2013).

These data therefore support an approach to lameness control that encompasses the life-cycle of the cow, starting during the heifer rearing period, with strategies that are lesion specific and age-specific, tailored to the type of lesions that are most prevalent on each farm. Understanding the motivation for farmers to implement change is critical for consultants (Leach et al., 2010). However, it would seem likely that with the growth and expansion of welfare audits globally, they will have little choice but to comply. Ultimately, producers that have succeeded in their control of lameness will become the best salesmen of prevention programs to the others that lag behind, and these producers will increasingly need an effective roadmap to expedite change.

Herd Risk Factor Oriented Strategies

Herd level risk factors for lameness have been studied in multiple countries and in a variety of production systems in recent years. A number of consistent findings have emerged from these studies. Factors which appear to be associated with lower lameness risk include; less time standing on concrete (Bell et al., 2009), deep bedded comfortable stalls (Chapinal et al., 2013; Cook, 2003; Dippel et al., 2009; Espejo et al., 2006; Rouha-Mulleder, et al., 2009; Solano et al., 2015), access to pasture or an outside exercise lot (Chapinal et al., 2013; Hernandez-Mendo et al., 2007; Popescu et al., 2013; Rouha-Mulleder, et al., 2009), prompt recognition and treatment of lameness (Barker et al., 2010), higher body condition score (Dippel et al., 2009; Espejo et al., 2006; Randall et al., 2015), use of manure removal systems other than automatic scrapers (Barker et al., 2010), use of non-slippery, non-traumatic flooring (Barker et al., 2010; Sarjokari et al., 2013; Solano et al., 2015a), use of a divided feed barrier (rather than a post and rail system), with a wider feed alley (Sarjokari et al., 2013).

It should be expected that routine professional hoof-trimming, access to a trim-chute for treatment and use of an effective footbath program would deliver improvements in lameness (eg. Pérez-Cabal and Alenda. 2014), but these effects are often confounded in associative observational studies (eg. Amory et al., 2006). It is also true that many poorly trained hoof-trimmers cause more harm than good, and many footbath routines are ineffective through poor design and management (Cook et al., 2012; Solano et al., 2015b). Similarly, several studies point to restrictive neck rail locations, high rear curb heights, and lunge obstructions as risk factors for lameness (eg. Chapinal et al., 2013; Dippel et al., 2009; Rouha-Mulleder, et al., 2009), however correct neck rail location and curb height is stall design specific and care should be taken in interpretation of these findings.

High Production and Low Levels of Lameness

While we know that Holstein cows are perhaps more susceptible to lameness (eg. Sarjokari et al., 2013), and there appears to be a genetic component to the development of DD, SU and WLD, with a link to higher milk production (Oikonomou et al., 2013), I do not believe failure is inevitable.

We had the opportunity to visit 66 high performance Wisconsin herds that have been implementing strategies to prevent lameness for over a decade (Cook et al., 2016). These herds had a mean herd size of 851 cows, were confinement housed in freestalls and produced more than 40 kg energy corrected milk per cow per day on average. The prevalence of clinical lameness averaged 13.2% - which would rival the degree of lameness identified in grazing herds (e.g. 8.3% as reported by Fabian et al., 2014), and mixed housing and grazing or organic management systems elsewhere (e.g. 16.5% in Amory et al., 2006; 17.2% in Rutherford et al., 2008). Interestingly, it is lower than the prevalence found in similar herds in the Midwest a decade or more ago

Management Characteristic	% Herds or Mean
% Sand bedded stalls (deep loose bedding including manure solids)	62 (70)
% 2-row stall layout pens (vs 3-row)	61
% Use of headlocks at the feedbunk	83
Milking Frequency (% 3 times a day)	67
% Use of rBST	67
% Solid floor (vs slatted)	100
% Rubber floors in freestall alleys	5
% Rubber floors in transfer lanes	15
% Rubber floors in holding areas	41
% Rubber floors in parlors	68
% Manual manure cleaning from the alleys	73
% Use of fans over the resting area	96
% Use of water soakers in the pens	79
% Allow access to the outside to roam	9
% Trimming at least once per lactation	88
% Trim cows at least twice per lactation	65
% Trim heifers before calving	49
Mean footbath frequency (milkings per week)	4.5
Mean cows per full time equivalent (FTE) worker	62

Table 2. Management characteristics of the high producing multiparous group cows in elite housed dairy herds in Wisconsin (from Cook et al., 2016).

(e.g. 22.5% in Cook, 2003; 24.6% in Espejo et al., 2006), suggesting that the overall degree of lameness in the region may be improving. Severe lameness was also uncommon at a mean of 2.5%. This average is lower than that found in the majority of previous freestall surveys (e.g. 5.3% in Barker et al., 2010; 16% in Dippel et al., 2009; 4.8% in Husfeldt et al., 2012). Thus it would appear that high performance can be compatible with acceptable lameness levels, if we manage cows correctly. Table 2 highlights some of the management characteristics of these herds pertaining to lameness management.

When examining the management strategies with high levels of adoption in Table 1, there are consistencies with the herd level risk factors documented previously. These herds use deep loose bedded stalls, have 2-row pen layouts with headlocks, have solid flooring with strategic use of rubber floors, especially around the milking center. Notably, these herds were not using rubber flooring in their pens to control lameness. They clean manure from the alleys when the cows are outside the pen, and have aggressive hoof care, heat abatement and footbath programs. Two thirds of herds use rBST and milk three times daily, and perhaps surprisingly, 9% let their high producing cows outside the barn strategically – not to graze, but to spend time away from concrete floors inside the barn. In a multivariate model, deep bedded stalls, pasture access and fewer cows per FTE worker significantly reduced the risk for lameness overall.

Lameness management will continue to be refined, but these herds prove that we know enough right now to implement positive change in the dairy industry and achieve acceptably low levels of lameness, even in cattle which may be inherently more susceptible.

A Structured Approach to Lameness Prevention

When troubleshooting lameness problems, I use a structured approach starting with locomotion scoring, lesion analysis and assessment of the routine hoof-trimming and lame cow surveillance program. It is essential that the hoof-trimming is a component of prevention rather

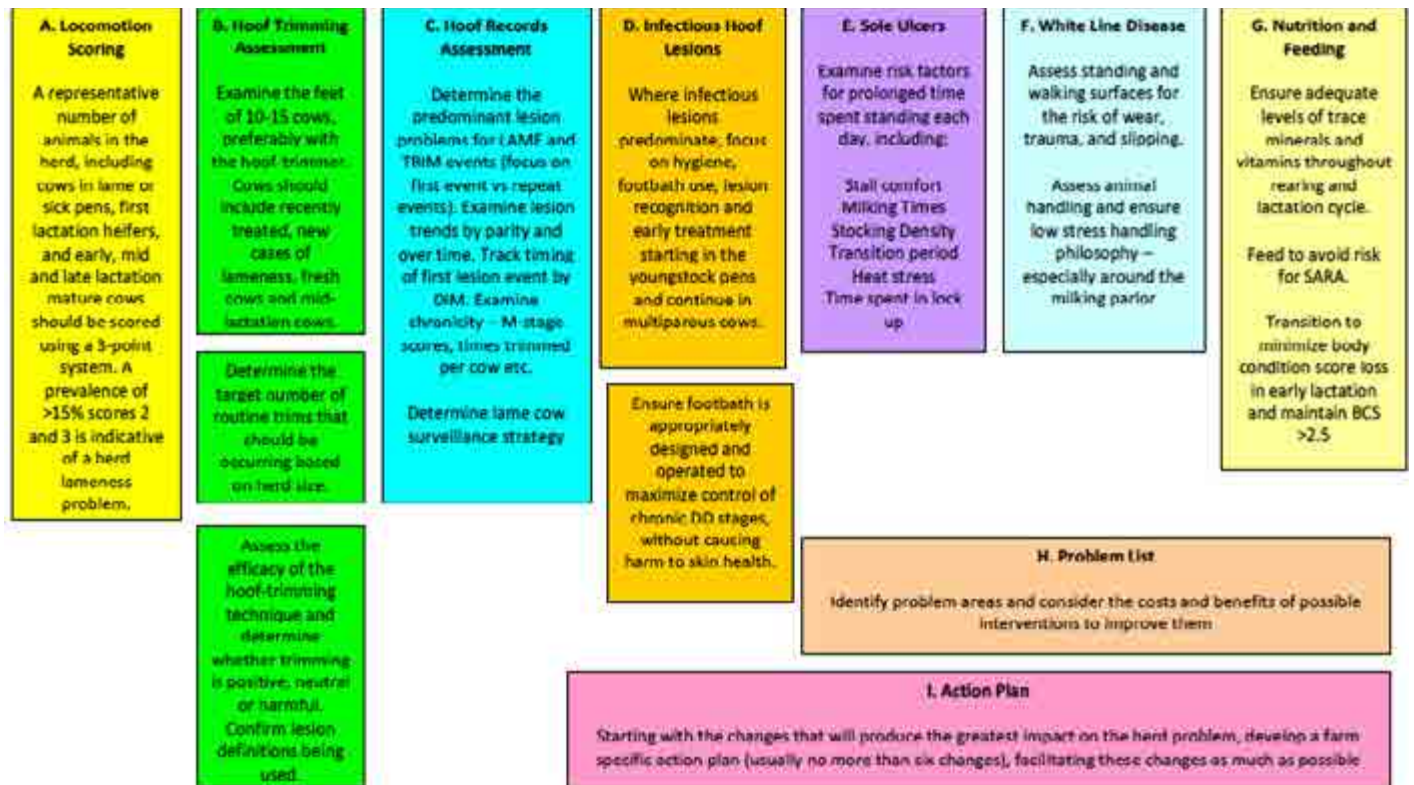


Figure 2. Herd lameness troubleshooting plan

than a risk factor in itself. I then examine the risk factors for each of the key hoof lesions and finish with a review of feeding practices. From this examination, we can create a herd specific action plan designed to maximize impact on the key hoof lesions on the farm.

For DD prevention, we focus on the early identification of acute lesions (before the cattle are lame) and prompt effective treatment, starting around breeding age in replacement heifer pens and continuing throughout the life of the animal, coupled with an effective footbath program to control the chronic lesions and hold them in check. Trace mineral supplements have a significant role to play, particularly during the rearing period. For SU prevention we target risk factors that extend daily standing times – stall design and surface cushion, stocking density, milking times, heat abatement and lock up time for management tasks. We optimize the transition period to maximize rest and reduce BCS loss in early lactation. Finally, for WLD control, we examine areas of the farm where flooring puts the cow at risk of slipping, trauma and excessive hoof wear, and watch workers to ensure low stress handling – especially around the parlor operation.

The overall approach is summarized in Figure 2. Each assessment results in a problem list which can then be used to develop a targeted action plan for the herd.

Conclusion

In this article, I have made the case, that while we still have knowledge gaps to fill in our understanding of lameness, the global crisis that we face with 1 in 4 cows walking with a painful limp can be solved by implementing our current knowledge targeted at the key hoof lesions; DD, WLD and SU. The challenge we face is one of creating a simple roadmap targeted at an individual producers most significant problems and motivating that producer to implement the changes necessary. Dairy producers that have already achieved success in lameness prevention will serve an important role motivating others to follow in their foot steps.

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Dairy Reproduction - the future

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Introduction

Dairy herd health management is undergoing a period of radical change worldwide. The drivers of this change are many. They include the massive increase in technologies to aid in dairy cow reproductive management, quota removal (within Europe) and the significant increase in herd/farm size. Following the removal of quotas in Europe there are ambitious plans to expand dairy output, this will be achieved by a combination of increased herd size and greater milk output per cow. This paper aims to identify some of these changes and emphasise those that can facilitate enhancements in herd health and reproductive management of dairy cows.

Herd fertility and data management strategies

Historically, the emphasis in veterinary medicine has focused on the individual cow affected with a clinical disease. However, about 30 years ago it was recognized that subclinical disease was the major cause of economic losses in dairy herds. The multifactorial origin of most subclinical diseases was examined during regular herd visits by veterinarians. This turned out to be effective in improving the overall health status of the herd, and hence profitability. This approach was called herd health management and has been implemented in veterinary education for at least 3 decades. Over the same time period, Internet and Communication Technology has emerged and integrated in herd health management to leverage the understanding of cow records. The way researchers have to leverage the power of Big Data has been at the center of attention ever since the publication trend that started around 2009 (Chen et al., 2012; Sagioglu and Sinanc, 2013). How to address these challenges will be the main scope for future research.

Techniques used for analysis and visualization of traditional dairy data are not adequate for Big Data. The volume, velocity, variety, distributedness and incremental nature of such data impose challenges on the traditional methods for data analysis.

Genetic strategies to improve reproduction

Traditional dairy genetic selection programmes in dairy producing countries up to the early 2000s selected predominantly for milk yield often at the expense of other dairy relevant traits, including fertility and health. Breeding programmes in the early part of this century started to include fertility (eg longevity and calving intervals) and health as part of the selection traits. Inclusion of these traits has served to reverse some of the earlier trends that gave rise to reduced fertility. Over the last 15 years it is now recognized that trends in both longevity (increased) and calving intervals (decreased) have improved. The major challenge for breeding programmes in terms of incorporation of fertility traits has been to develop phenotypes that have high heritability. For example many fertility traits have typically only low heritability estimates (e.g., 0.1, compared with many growth and carcass traits where the heritability is 0.25-0.5). A second major issue for many fertility traits is to have easily measured phenotypic traits or genomic markers (SNPs) that correlate to appropriate fertility traits.

The EU funded project genotype plus environment has amongst its many objectives the identification of new novel milk-based phenotypes that may be used as predictors for the traditional, but difficult to measure, record and select traits such as conception rates and uterine health

(www.gpluse.eu). A key aim of the project is to develop novel milk based traits that correlate and predict health and fertility traits in dairy cows. The strategies being used include the measurement of Mid-infrared spectra (MIR), metabolites and glycans on the IgG fraction of milk. These phenotypes will be used to help identify novel genomic markers (SNPs) for fertility and health traits, this approach will allow for improved selection strategies in the future.

Furthermore recent work in University College Dublin has led to the development of glycan markers for uterine health. This has been developed into a patent application (PCT/EP2014/068734: "Methods for predicting, diagnosing or monitoring infections or conditions"). Indeed milk-based glycan markers have also been developed that can identify cows having retained placental membranes (Santoro et al., 2016). Such biomarkers that are easily measured in milk would allow animal breeders select for cows with a propensity for improved uterine health and therefore move towards cows that would have increased fertility.

Nutritional strategies to improve reproduction

Modern dairy cows have been predominantly selected for a high milk yield in early lactation that is associated with a very high capacity to mobilize body reserves during this period. Calculations showed that cows can produce as much as between 120 and 550 kg of milk per lactation from body reserves (Tamminga et al., 1997). Most cows can cope with this metabolic load that is defined as: 'the total energy burden imposed by the synthesis and secretion of milk, which may be met by mobilization of body reserves'. Metabolic stress however is defined as 'the amount of metabolic load that cannot be sustained by this mobilization, leading to the down-regulation of some energetic processes, including those that maintain general health (Knight et al., 1999). Hence, the 'over' mobilization of body reserves during the period of NEB is a key factor for disease susceptibility in modern dairy cattle.

Management strategies for transition cows are mainly focused on helping the cows to cope with the metabolic load by optimizing health, minimizing stress (e.g., by minimizing the changes in group or ration), stimulating dry matter intake and immune function. Furthermore, application of diets specifically designed to improve fertility by counteracting mechanisms related to the negative energy balance (NEB) or by supporting a specific pathway that is necessary for successful fertility, has always been a very attractive way to circumvent the impairment of reproduction during early lactation.

Controlling infectious diseases

Veterinarians managing fertility in dairy herds should regularly evaluate the herd health status for pathogens known to compromise reproductive efficiency. Infections with pathogens like *Leptospira hardjo*, Bovine Virus Diarrhoea or Herpes viruses are known to reduce conception rates, while infections with *Neospora caninum* and emerging viruses like the Bluetongue virus may cause foetal losses and abortions. Bovine Herpes Virus 4 is reported to have a tropism for endometrial cells which is therefore suggested to be especially monitored and controlled in herds suffering from uterine diseases (Donofrio et al., 2007). Besides continuing careful monitoring, appropriate biosecurity plans eventually including vaccination protocols should be implemented to prevent the introduction of new agents into the herd and to prevent eventual spread within the herd (Sanderson and Gnad, 2002).

Of special interest among infectious diseases, is the minimization of uterine diseases. In cattle, bacterial contamination of the uterus is ubiquitous at parturition. However, the latter does not automatically imply the establishment of uterine disease and subsequent fertility problems. Despite the fact that several papers have been published aiming to come to a general agreement about the definitions of postpartum uterine diseases based on mainly clinical symptoms (LeBlanc et al 2002; Sheldon et al., 2006), there is still a lot of confusion about these

definitions among practitioners. The latter gives rise to a wide variety of preventative and curative treatment protocols being applied in the field, many of which are not scientifically proven to be efficacious. Recent literature underlines the high incidence of subclinical endometritis in high yielding herds. Diagnosis of this impairment is based on intra-uterine sampling for cytology, which is not routinely done at the moment. Therefore, recently we reported the use of the cytotape that allows sampling during insemination and facilitates for example profiling in repeat breeder cows (Pascottini et al., 2015). The generally accepted necessity to minimize the use of antibiotics in cows should be extended to treatment of uterine infections. It is important to determine the risk factors for the different uterine diseases, and design prevention and control programmes to reduce the incidence of disease.

Use of precision livestock farming

Oestrous detection

Successful reproductive performance based on detection of oestrous behaviour implies the need to accurately detect oestrous onset in the majority of cows, and then inseminate 4 to 16 hours later. This led to the common practice of breeding cows according to the am-pm rule. This requires that cows are observed for oestrus 5 times per day, and those commencing oestrus in the morning get inseminated that evening and those commencing oestrus after 12.00 noon are inseminated the next morning (onset of oestrus defined as the first observation period where the cow stands to be mounted). This approach has served well for herds prepared to invest the time and effort into good and accurate oestrous detection. However, it requires a significant commitment of labour, good cow identification and personnel trained in detection of oestrus in cows.

Aids to oestrous detection

To achieve high submission rates to AI, which are critical to achieve a 365-d calving interval in seasonal calving herds, requires an effective, practical means of identifying each cow in oestrus. Both the physical activity and mounting activity induced by increased oestradiol production during the preovulatory follicular phase can be monitored in various ways. Visual observation of mounting activity for 20 min 3 to 5 times per day results in 90 to 95% submission rate (Diskin and Sreenan, 2000) but is laborious and time consuming. In high-yielding dairy cows, the percentage of cows that display standing to be mounted by other cows has decreased, leaving it more difficult to detect oestrus. Roelofs et al. (2005) found that only 58% of cows were observed in standing oestrus. This, in turn, decreases submission rate to AI and thereby contributes significantly to reduced reproductive efficiency (Diskin, 2008).

Sensors for oestrous detection

Over the last 2 decades various systems for automation of oestrous detection have been developed to various degrees of success.

Pressure sensors (Heat Watch). The characteristic oestrous behaviour of standing to be mounted can be monitored through the use of systems such as the electronic device HeatWatch, scratch cards (e.g., Estrotec; Rockway Inc., Spring Valley, WI), colour ampoules (Kamar Products Inc., Zionville, IN), vasectomized bulls fitted with a chin-ball marker, or the use of tail-painting methods (Diskin and Sreenan, 2000).

Activity monitors (eg Heat time, dairy master etc). Attempts have been made to monitor changes in physical activity to predict oestrus using automated systems. The pedometer, attached to a leg, detects an increase in the number of steps taken per hour during oestrus (e.g., S.A.E. Afikim, Kibbutz Afikim, Israel; Holman et al., 2011), whereas the use of a neck collar (e.g., Alpro; DeLaval International AB, Tumba, Sweden; Peralta et al., 2005) identifies increased physical activity (walking, mounting, getting up and lying down) expressed as an activity cluster (AC). A recent study, using the neck collar activity monitor Heatime (SCR Engineers Ltd., Netanya, Israel), identified that the odds of an AC being in a preovulatory follicular phase rather than a luteal

phase improved by 29% for every 1-unit increase in peak activity and by 91% for every 2-h increase in duration of an AC (Aungier et al., 2012). Heatime could alert the farmer as to when the AC started (when the cows next enter the milking parlour). It can identify the optimum time to AI, which is during a 12- to 18-h window before the predicted time of ovulation. This optimum time is, on average, between 9 and 15 h after the AC has been triggered (Aungier et al., 2015).

Endocrine profiling. Delaval have recently developed in-line measurement systems to detect metabolites and progesterone concentration in milk (Friggens et al., 2008). Using algorithms progesterone profiles may be de-convoluted to predict oestrous events. However, to date this technology is still relatively expensive which is limiting its adoption. In time this will likely be an important approach to managing reproduction in large dairy herds.

Oestrous synchronization and ovulation synchronization

Traditional oestrous synchronisation methods were designed to synchronise oestrus, but at best still requires observation of oestrus to optimize timing of mating and pregnancy rates. The use of 2 injection of prostaglandin 11 days apart in heifers can work well with FTAI at 72 and 96h or alternatively at 72h, and then intensively observe for oestrus for a further 3-4 days and inseminate those late coming into oestrus, in response to standing oestrus (using the am-pm rule).

Ovulation synchronisation programmes were designed to facilitate use of AI in herds without significant investment of time and labour into oestrous detection. These were developed from the early 1990s onwards (Pursley et al., 1995). They are more appropriate to large non-seasonal herds where calving to calving intervals are somewhat less relevant to economic performance of the herd and often calving intervals are allowed to extend beyond 400-420 days. The major issues for a basic OVSYNCH programme is that conception rates to a single round of OVSYNCH are approximately only 30%; and in a European context are relatively expensive. Strategies to improve pregnancy rates have been developed (e.g., Double OVSYNCH and PRESYNCH OVSYNCH; Herlihy et al., 2012) that are acceptable in many US herds, but incur substantial costs in terms of time and drug costs that leave their use questionable in European dairy herds.

Progesterone synchronization programmes can reduce the time associated with the breeding programme and are often more effective for certain categories of cows (McNally et al., 2014).

Pregnancy Detection

Direct methods of pregnancy detection

Various methods are available to determine pregnancy status, these include return to oestrus, rectal palpation of the reproductive tract and ultrasound scanning to observe the reproductive tract. In practice return to oestrus is fraught by the vagaries of oestrous observation, so currently most pregnancy detection in cows is carried out by ultrasound scanning of the reproductive tract to detect the presence or absence of the early embryo and foetal fluid. Using this method, pregnancy status is generally determined from day 28. This method while routinely used, is too late to allow rebreeding at the optimal time for non-pregnant cows.

Indirect methods for pregnancy detection in dairy cows

Indirect methods for early pregnancy diagnosis use qualitative or quantitative measures of hormones or conceptus-specific substances in maternal body fluids as indirect indicators of the presence of a viable pregnancy. Commercially available indirect methods for pregnancy diagnosis in dairy cows include milk progesterone tests and tests for pregnancy-associated glycoproteins (PAGs) in blood or milk.

Progesterone assays are reasonable as a non-pregnancy test on day 21 (Nebel, 1988). However, it is inaccurate as a test for pregnancy as

reversion to low progesterone in non-pregnant cows is highly variable due to early embryonic losses. It has been tried commercially, but has not survived due to these problems. The in-line version developed by Delaval (herd navigator) allowing repeated sampling over time has potential if the costs of repeated analyses can become competitive.

PAG measurement is a viable method of determination of pregnancy status of dairy cows (Cordoba et al, 2001), however, accuracy of PAG detection is only good after day 35 / 40. Interference may also occur due to PAG carry over from previous pregnancy for 40-50 days giving rise to a risk of false positives. It may also give false positive results after embryo loss.

Recent work from the UCD School of Veterinary Medicine has led to the development of a test based on glycan diagnostics using the IgG fraction in milk. This technology can detect pregnancy status from as early as day 16 and has led to a priority patent filing (filed 17 November 2015; UK Patent Application No.1520248.4).

The male side of the story

Although most evidence suggests the pressure on reproductive efficiency in modern dairy herds is primarily related to the high producing females, it is obvious that the role of the male should not be forgotten. While veterinary practitioners often overlook the importance of this side of the coin, farmers often blame issues like sperm quality and the expertise of the AI-technician, often because it is human nature to prefer blaming someone else instead of being critical against personal shortcomings. The success of an insemination depends amongst other factors on the deposition of appropriate numbers of sperm with a good fertilizing capacity at the appropriate site in the reproductive tract at the appropriate time in relation to ovulation. The fertility potential of an artificial insemination dose is a function of the quantity, quality, and health status of the semen contained therein. It is the task of the AI-industry to continue to maintain intense quality control programmes to ensure cryopreserved semen doses released for sale are disease free and meet the above mentioned criteria.

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Therapeutics & Pharmacology

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“Pharmacology” and “therapeutics” represent broad subjects: hundreds of books and journal articles have been published on them, even within the more limited realm of cattle pharmacology and therapeutics. By describing what I find intriguing and challenging about bovine pharmacology and therapeutics, I hope to highlight some important aspects of cattle pharmacology and to provide some food for thought about therapeutic decision-making in cattle.

Our brains work in interesting and predictably inaccurate ways, so we must compensate by being systematic in how we make therapeutic decisions.

More and more evidence suggests that our brains have two major streams: fast and slow, or intuitive and analytical, or System 1 and System 2. Experts, including all but new veterinary graduates, can be successful and accurate using the fast and intuitive system to approach diagnoses and medical management, but they can also be wrong. Being aware of the pitfalls of both types of thinking is critical to approaching therapeutic decision-making in an evidence-based and defensible manner.

How veterinarians make decision about drugs has not been extensively examined or described in the literature, but it is a fascinating aspect of therapeutics.

As veterinarians, you should aspire to justify and validate your clinical decisions, especially if your metacognitive muscles are underdeveloped. When it comes to drug decision-making, the treatment effect of a drug regimen (regimen being drug, dose, route, frequency, and duration) should be an important input as to whether and how to use drugs. It is not, however, the only input, and all the characteristics below must be balanced for adequate, accurate, and defensible drug choices. Drug choice is not always addressed as a process of clinical reasoning, so I encourage deliberate consideration of the following inputs:

“Alteration in the animal” – what has the disease or condition altered in the patient that is amenable to intervention, or what intervention in a normal animal might result in a desired physiological alteration? Are you guessing at the alteration, or do you have enough evidence to make at least a presumptive diagnosis?

“Therapeutic goal” – what is the specific desired outcome? Can it be quantified so it can be later examined to compare predicted with actual outcome to assess efficacy (see also “Indications of therapeutic success” below).

“Drug Options,” including mechanism of action and variability of response – what drugs might result in the desired therapeutic goal in the face of the particular alteration in the patient, how do they work, and how likely are they to work? No drug is effective in 100% of animals, so what is your best estimate of the treatment effect?

“Common or significant adverse effects” and “Contraindications” – are there potential adverse effects that may limit options in some animals, for example, because of co-existing conditions? Does the injection site reaction lead to significant effects on meat quality?

“Legal issues” – are there legal limitations on the drug options for the given therapeutic goal? If you are in academic practice or you mentor budding veterinarians, do you model good decision-making by weighing legal issues as or more heavily than efficacy when choosing between alternatives? Consideration should be given here also to ethical selection of drugs and regimens: even if legal, does recommending this regimen represent a conflict of interest, or would it lead to inappropriate masking

of animal conditions or behaviors, or could it result in an important public health consequence?

“Patient or Organism factors that alter regimen” – is there a characteristic of the treated animal such as disease of the organs of clearance that could alter the regimen selected? If bacterial infection is the alteration, are there resistance mechanisms present that might lead to failure or the requirement of a different dose?

“Indications of therapeutic success” – how will you know that the treatment worked? And what are your plans for monitoring outcomes to ensure that your treatment choices are as effective as you expected?

“Sources of information” – what information do you use to inform your drug decisions, and how do you acquire and critically evaluate that information?

These inputs to making drug decisions can be deliberately probed either on your own or with colleagues, exemplifying critical thinking and resulting in better decisions. Some example questions could include: Do you unintentionally limit your drug decisions by making a drug choice initially based on withdrawal time rather than efficacy? Do you always include a way of evaluating treatment outcome in your medical records? Can you defend your ultimate drug choice based on all its characteristics?

Predictions about the treatment effects and side effects of drugs are not always accurate, so scientifically valid investigations are needed to assess those predictions.

The essence of the principles of evidence-based veterinary medicine is that evidence varies in strength and quality, and we must examine both in our search for the truth about what interventions are likely to result in a measurable impact on disease outcome. Not all “Sources of information” are equal, and a systematic approach to acquiring and evaluating evidence is necessary. The general approach has been described in various ways, but the common steps are to use the knowledge gap (what do you need to know to make a decision in a particular case) to:

- Create a clinical question, with the commonest approach being the PICO-based question: patient and its problem, intervention, comparison (which may be as simple as compared to doing nothing), and outcome.
- Search for evidence to answer the question.
- Appraise the evidence for quality in terms of validity and applicability to the question.
- Apply the evidence to the question to make a decision.

There are two important outcomes of this approach: (1) a search for and review of all and of the most recent data and (2) critical appraisal of those data. By critically appraising, one determines the actual value of the data, rather than just assuming that all data are equal. Evidence pyramids have been described that attempt to weight data more heavily that come from systematic reviews of the literature or that are the result of randomized and controlled clinical trials, but the essential point is that not all data are equal. Data that are likely to be biased or for which bias cannot be assessed are of less value and should perhaps not be applied.

A third outcome of evaluating the evidence is a better characterization of the actual treatment effect associated with a drug or drugs. Treatment effect is the difference between the disease outcome with and without treatment, sometimes delineated as Number Needed to Treat or NNT. However, it can also be as simple as finding, for example, that the treatment effect of even a highly effective antibiotic on bovine respiratory disease outcomes averages approximately 50% (24% of untreated control animals vs. 71% of antibiotic treated animals, with an outcome of recovery rate). Predicting drug efficacy in this way is much different than what might be a more common, at least to the novice, notion that untreated animals never recovery and treated ones always do (100% recovery rate)!

But what if a systematic review of the literature or knowledge summary does not exist, or what if there are no in vivo or even in vitro data available? Clinical experience is often touted as the next best source of evidence. Veterinarians must be aware, however, that using clinical experience inevitably brings bias to the table, with the most common errors being a result of a lack of a comparison group, confounding by indication, loss to follow-up, and biased outcome measurements.

Cattle are food animals, so the disposition and effects of drugs in cattle in terms of food safety and food quality are important inputs to therapeutic decision-making.

As mentioned above in the "Legal issues" input in drug decision-making, cattle veterinarians know that they have important responsibilities in keeping food products from cattle safe from harmful concentrations of drugs (or withholding animals treated with dangerous drugs from the food supply). This may be as simple as following the labeled withdrawal time for a particular product, but it may be as complicated as extrapolating a withdrawal time from the scientific literature or having a difficult conversation about the ethics of a particular drug and regimen. Other considerations globally related to the importance of food safety considerations include the veterinarian's responsibility in understanding access to drugs, the drug approval process in your jurisdiction, your assurance of drug safety and purity (particularly of importance for drug compounded from bulk active pharmaceutical ingredient), and so on.

In addition to food safety, veterinarians can play an important role in contributing to food quality, for example, in the selection of drug products that are less irritating or that can be administered in areas of the animal that are of lower economic value. Beef Quality Assurance programs include best practices on injection sites because of the potential impact on tenderness, and veterinarians can lead the way in making therapeutic choices that are likely to be effective AND to result in a high quality food product.

Science does not always trump politics.

Even when veterinarians use evidence and systematic decision-making processes, other factors can intervene in treatment decisions, because bovine veterinarians today are at the intersection of science and politics. Salient examples include (1) the impact of use of antibiotics in animals on the global public health crisis of increasing antibiotic resistance, (2) animal management practices and procedures that result in pain or affect animal welfare, and (3) the potential for conflict in the desire to feed growing populations by maintaining or creating sustainable food production while minimizing environmental impacts (e.g., climate change, bacterial or chemical contamination) in the face of uncertainties in the currently available data and in the ability to predict the trajectory of any of the inputs or outcomes. It is fair to say that all of these examples could be addressed to some degree by analyzing the available scientific data. However, the scientific data may not be unequivocal in its ability to provide evidence-based recommendations for future behavior given the complicated and broad nature of these issues. More importantly, even well-designed unambiguous data about antibiotic resistance, animal welfare and pain, and sustainable food production may not be persuasive given the underlying moral facets of these topics. And moral arguments often lead to political action.

So what should the bovine veterinarian do? In my experience, advocacy whether local, regional, national, or international can have an impact, as can engagement in veterinary professional organizations. Importantly, veterinarians must not lose heart. Fringe opinions may get the attention, but often the people actually doing the work of governing and regulating are trying to listen to the scientists and the health professionals. And when health professionals disagree, best practices in conflict resolution and mediation can result in closer alignment in solving the problem.

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Overview of Metabolic Disease: Impact of Energy, Protein and Mineral Issues on health and immunity of the periparturient cow

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Most of the metabolic diseases of dairy cows—milk fever, ketosis, retained placenta, metritis, and displacement of the abomasum—occur within the first 2 wk of lactation. The etiology of many of these metabolic diseases can be traced back to problems in three basic areas. These are energy and protein balance, hypocalcemia, and immune dysfunction. The incidence of both metabolic and infectious diseases is greatly increased whenever one or more of these physiological functions are impaired.

Energy considerations for periparturient cows

The optimal energy content of the diet fed to the cow prior to calving is a controversial topic. The trend for many years was to feed the cow a diet with a higher energy concentration than she requires for most of the dry period. The rationale for this strategy included: 1. Adaptation of rumen microbes (Huntington, et al., 1981) and rumen papillae (Dirksen and Liebich, 1985) to the type of high-energy diet the cow will receive in lactation; 2. Maximizing DMI prior to calving as this may be correlated to DMI in early lactation (Grummer, 1995); and, 3. A diet with higher energy concentration can compensate for the decline in dry matter intake commonly observed in the final week of gestation. This might prevent the cow from going into negative energy balance just before calving. Unfortunately, this strategy is based on the premise that the dry matter intake depression just before calving would be similar in cows fed low and high energy diets during the final weeks of gestation, which may not be true (Beever, 2006, Grummer, 2008).

Increasing the non-fiber carbohydrate content of a pre-partum ration will often increase DMI across the entire three weeks of the close-up period (Holcomb, et al., 2001, Minor, et al., 1998). Increasing the energy content of the pre-partum diet has also been reported in some studies to result in lower blood non-esterified fatty acid (NEFA) or β -hydroxybutyrate (BHBA) concentrations and lower liver triglyceride accumulation (Doepel, et al., 2002, Mashek and Beede, 2000, Vandehaar, et al., 1999). However, this approach does not generally result in increased DMI after calving or increased milk production. There is also indirect evidence that supplying energy to the cow in excess of her needs may render her tissues less sensitive to insulin (Holtenius, et al., 2003; Dann, et al., 2006). Displacement of the abomasum is also promoted whenever the NFC content of the diet is increased, unless great care is taken to maintain physically effective fiber content of the ration (Coppock, 1974). When higher energy diets are fed more than 5-6 weeks prior to calving the cows may also gain excess body condition. Data also suggest increasing energy density of pre-partum diets actually leads to a greater decline of DM and energy intake in the 2-4 days before calving, i.e. the number of kg/d by which DMI is depressed just before calving may be greater in cows on the higher energy diets (Hayirli, et al., 2002, Ingvarsen and Andersen, 2000, Minor, et al., 1998, Olsson, et al., 1998)

Retrospective evidence suggests those cows suffering a bigger change (# kg) in DMI just before calving will be at greater risk of energy related disease (Grummer, et al., 2004). Studies on periparturient immune function also suggest those cows suffering the greatest decline in feed intake just before calving are most immune suppressed, leading to increased incidence of metritis and endometritis (Hammon, et al., 2006, Urton, et al., 2005). If DMI during the week before and after calving is critical to avoidance of fatty liver-ketosis and improved immune function—how do we best feed the cow to achieve this?

In the last ten years a number of dairies have adopted the strategy to

shifted to feed a lower energy diet, which utilizes straw or mature hay as a major component of the diet. Typical steam-up diets often provide ~ 1.56 Mcal NE_L / kg. The lower energy diets typically provide about 1.35 Mcal NE_L / kg diet. It should be noted that the typical DM intake of the low energy diets across the three weeks before calving will be 1-2 kg / day lower than with the high energy, higher starch diets. However, it must be emphasized that the low energy diets still provide more energy to the cow than she requires. The High energy diets provide 4-6 Mcal / day more than the cow requires. This seems to reduce their desire to eat the last days of gestation and first days of lactation. The high straw diets do provide another major advantage— a lower incidence of displaced abomasum is observed in herds fed these diets. Studies have been less successful in demonstrating these diets improve milk production or reduce the incidence of ketosis and other disorders. Perhaps it is time to focus more on the fresh cow diet as a cause of many of the fresh cow's energy problems?

Negative Protein Balance in Fresh Cows

The fresh cow is also in negative protein balance shortly after calving. The typical cow will lose 16 kg body protein during the first 2 weeks of lactation. Much of this body protein is being used to support the amino acid and glucose requirements of milk production (Paquay et al., 1972). Therefore in many respects the dairy cow in early lactation is in a physiological state comparable to that of humans and rodents with prolonged protein-calorie restriction. Glutamine is the most abundant free amino acid in human muscle and plasma and is utilized at high rates by rapidly dividing cells, including leucocytes, to provide energy and optimal conditions for nucleotide biosynthesis. As such, it is considered to be essential for proper immune function. Interestingly, plasma glutamine does not seem to be depressed in the dairy cow at calving – when body fat mobilization is rapidly increasing and presumably protein catabolism would also be increasing rapidly. However plasma glutamine does decrease as the cow progresses into the early weeks of lactation (Zhu et al., 2000, Meijer et al., 1995). Cows are in negative protein balance for 1-2 weeks after calving. Meijer et al., 1995 found that plasma levels of many of the essential amino acids fell drastically after calving and took about 10 days to 2 weeks to return to baseline levels. Plasma concentrations of glutamine, the non-essential amino acid used as an alternative energy source by many cells, including immune cells, did not return to normal levels until the cows returned to positive energy balance some 3-5 weeks into lactation.

Does the relative calorie-protein deficiency of the early lactation cow impact her immune response? In humans, protein-calorie restriction has severe effects on cell-mediated immunity. There is often widespread atrophy of lymphoid tissues and this can cause a 50% decline in the number of circulating T-cells. Surprisingly antibody responses are intact and phagocytosis of bacteria is relatively normal. However destruction of bacteria within the phagocytes is impaired (Roitt, 1991). Moderate calorie restriction (with or without a severe reduction in fat intake) has been used to ameliorate the effects of auto-immune disease, presumably by reducing cell-mediated immune responses (Leiba et al., 2001).

In mice, malnutrition leads to immune dysfunction with greatly increased morbidity. Mice fed protein deficient diets and experimentally infected with parasites had worm burdens that were significantly higher than in mice fed adequate protein (Boulay et al., 1998). C57Bl/6 mice fed a low-calorie diet demonstrated a marked reduction in T-dependent-antigen-specific lymphocyte proliferation and antibody response when compared to mice fed ad libitum. The depressed lymphocyte response seen in calorie-restricted animals is attributed to a defect in both the macrophages and T cells in antigen processing, presentation, and/or proliferation (Christadoss et al., 1984).

We know very little about the effect protein/energy restriction might have on bovine immune function. One small study examined the effect of protein/energy restriction for 133 days on nine cows infected

with tuberculosis. Dietary restriction did not result in any significant reduction in skin sensitivity to PPD, in vitro production of IFN-gamma, or lymphocyte blastogenesis. The number of circulating BoCD4+ cells and B cells were similar in both the malnourished and the control cattle. However, significantly lower numbers ($P < 0.01$) of circulating BoCD2+ cells, BoCD8+ cells, WC1+ gamma delta T cells and ACT2+ cells were found in the malnourished cattle (Doherty et al., 1996).

Management factors that will greatly influence the degree of negative energy and protein balance a cow will experience include number of cows in a pen (overcrowding), bunk space (optimal is 30 inches / close-up cow and perhaps also in fresh cow pen), number of "beds" for cows, movement of cows in and out of pens, timing of movement and number of moves in the transition period.

What is the energy cost to mount an immune response?

Little to no work has been done to examine this issue in cattle. However, in humans suffering from severe infection causing sepsis (various degrees of fever, increased WBC count, and acute phase protein production), the resting energy expenditure (determined by indirect calorimetry), increased progressively over the first week of the infection to around 40% above normal and was still elevated 3 weeks from the onset of illness. As an aside; over a 3-week period patients lost 13% of their total body protein (Plank and Hill, 2000).

No such measurements have been reported for cattle. However if we are allowed to extrapolate and speculate we can go through a few calculations. Maintenance energy for a 600 kg dairy cow is approximately 9.7 Mcal Net energy / day. If the cow must also increase energy expenditure 40% to mount an inflammatory response the energy requirement increases by nearly 4 Mcal / day. This is roughly equivalent to a requirement that the cow consume an additional 2.4 kg of diet (assuming a diet that provided 1.65 Mcal NEI/kg). Can the periparturient cow, already in negative energy balance, be expected to successfully mount a rapid immune response? If she is in fact in negative protein balance as well, will her immune system produce the immunoglobulins and acute phase proteins necessary to fight an infection while it is still in the initial phases to prevent it from escalating to a severe clinical infection?

Hypocalcemia – impact on cow health

Total blood Ca concentration in the adult cow is maintained between 8.5 and 10 mg/dL (2.1 and 2.5 mmol/L). Nearly all cows will experience some degree of hypocalcemia at the onset of lactation- the severity and duration of the hypocalcemia experienced depends on the integrity of the cow's Ca homeostasis mechanisms. Typically, the nadir in blood Ca concentration occurs 12- 24 h after calving. Surveys in the USA suggest around 5% of cows will develop milk fever each year, and the incidence of subclinical hypocalcemia – blood Ca values between 8 and 5.5 mg/dL (2 and 1.38 mmol/L) during the periparturient period – is around 50% in older cows (Reinhardt et al., 2011). Cows with sub-clinical hypocalcemia mobilize more body fat resulting in higher blood Non-esterified fatty acid (NEFA) concentrations, increasing the risk for ketosis and displaced abomasum. Hypocalcemia reduces rumen and abomasal motility increasing the risk of abomasal displacement. Hypocalcemia also reduces contraction of the teat sphincter muscle responsible for closure of the teat orifice after milking, thus increasing the risk of mastitis. Hypocalcemia is accompanied by reductions in intracellular endoplasmic reticulum and mitochondria stores of Ca in blood mononuclear cells. This impairs immune cell response by reducing the release of the "second messenger" Ca into the cytosol from intracellular stores in response to an activating stimulus (Kimura et al., 2006). Martinez et al., (2012) found that sub-clinical hypocalcemia impaired many aspects of neutrophil function and cows with sub-clinical hypocalcemia had higher blood b-hydroxybutyrate concentrations than normocalcemic cows. They also

concluded the relative risk of developing metritis increased by 22% for every 1 mg/dL decrease in serum Ca below 8.59 mg/dL. Sub-clinical hypocalcemia increases the risk for fatty liver development and reduces fertility of the cow (Chamberlin et al, 2013). Milk fever and subclinical milk fever should be considered gateway diseases that greatly reduce the chance for full productivity of a cow in the ensuing lactation.

Why does calcium homeostasis fail in some cows?

In most cows, the sudden exodus of Ca from the blood to support milk production is successfully met by reducing urine Ca excretion, increasing removal of Ca from bone fluid and bone crystals, and extraction of Ca from the diet- by both passive and vitamin D dependent transport mechanisms.

At one time it was thought milk fever occurred as a result of failure of the parathyroid glands to recognize and respond to the hypocalcemia induced by the onset of lactation. However, it was later discovered that cows developing milk fever have very high concentrations of PTH in their blood. In a landmark study, Martig and Mayer (1973) were able to demonstrate that the response of late gestation cows to exogenous PTH (which should cause an increase in blood Ca concentration) was diminished compared to the response elicited by PTH administered to cows in lactation, but they were not able to discern the cause. In severe cases of "relapsing" milk fever (cows relapsing and becoming recumbent again some hrs after the typical intravenous calcium treatment), it was observed that the secreted PTH in these cows fails to stimulate production of 1,25(OH)₂D to the same extent it does in cows with less severe milk fever (cows that require only a single intravenous calcium treatment to effect a recovery). This again suggested the periparturient cow's tissues were temporarily refractory to PTH stimulation (Goff et al., 1989). Experimental studies have identified a number of factors that interfere with Ca homeostasis in the cow. These factors are discussed below along with measures that can be taken to overcome their effects.

HIGH DIET CATION-ANION DIFFERENCE

Elegant work by Norwegian researchers Ender and Dishington in the 1970's demonstrated reducing the dietary cation-anion difference (DCAD), defined as the difference in the number of milliequivalents of cations (primarily K and Na) and anions (primarily Cl and S) in the pre-calving diet, improved Ca homeostasis at the onset of lactation. Block (1984) was able to confirm and extend this observation. Goff and Horst (1997) demonstrated high dietary K induced a metabolic alkalosis and found the risk of developing severe periparturient hypocalcemia was greatest in those cows in a state of metabolic alkalosis. Similar results were observed when Na was added to the prepartum diet. A recent study fed late gestation cows a High DCAD, alkalinizing diet or a Low DCAD, acidifying diet and treated the cows with synthetic exogenous PTH. The cows fed the alkalinizing diet had a greatly diminished response to the PTH compared to cows fed the acidifying diet. Their kidneys did not produce as much 1,25(OH)₂D and serum Ca did not rise as quickly (Goff et al., 2014). The authors speculate the tertiary structure of the PTH receptor is altered during metabolic alkalosis, reducing its affinity for PTH and resulting in a state of pseudohypoparathyroidism (Figure 2B). In many cows, despite the fact that bone and kidney cells are exposed to very high concentrations of PTH at the onset of lactation, they respond only poorly to the PTH. Addition of anions to a diet to counteract cations in the diet of a cow reduces the alkalinity of the blood and restores tissue responsiveness to PTH at the onset of lactation. Most studies have found physiological functions stimulated by PTH, such as osteoclastic bone resorption and production of 1,25(OH)₂D, were enhanced in cows fed diets with added anions. However, not all studies demonstrate an increase in osteoclast activity when cows are fed anionic diets.

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Bovine Tuberculosis Eradication in Ireland

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The Irish bovine tuberculosis (bTB) eradication programme, driven by production losses in cattle, human health problems and a desire to trade in live bovine animals, commenced in 1950 and by 1962 was compulsory nationwide. The programme ensures that production losses in cattle and human health concerns are no longer an active issue, and that Ireland may avail of trading opportunities, which expanded post 1965, as European trading conditions for live animals were met. Unlike in those countries, which have succeeded in eradicating bTB Ireland has a wildlife species (*Meles meles*), in which TB is endemic, sharing the same environment as bovines. Considerable research effort has been devoted to determining the contribution of wildlife to the Irish bTB problem and in developing a viable long-term solution. Ireland has set a goal of bTB eradication in 2030 and intends with strict adherence to testing, controls and biosecurity, together with continued measures to address wildlife TB to achieve that target.

Introduction

An account of the success of the bTB eradication programme in Ireland was written fifteen years after its commencement. Fifty years later we look again at progress made.

Losses due to overt disease in cattle, human health problems caused by *M. bovis* and trading requirements, with the British seeking attested store cattle from Ireland, led to the decision to commence the Irish bTB eradication programme. A bTB scheme had commenced in Britain in 1935. British research and experience in the conduct of the tuberculin test informed the Irish programme.

In 1964 Directive 64/432/EEC, the 'trading' Directive governing trade in live bovine animals within, between and to Member States of the European Economic Community had been adopted. By 1965 Ireland, although not in the EEC, had no herds of unknown disease status and on at least one occasion during the previous 11 years, all Irish herds either had individually achieved Officially Tuberculosis Free (OTF) status or been designated infected thus fulfilling the primary conditions to allow Ireland to take advantage of possible trading opportunities opening up within Europe. The downward trend in bTB was expected to continue towards final eradication. Instead, the eradication programme stalled with ~30,000 animals failing the tuberculin test and being removed annually (Figure 2). The veterinary dispute in 1975/76 and again in early 1985 curtailed the testing programme while ongoing, but seemed to have no lasting impact.

In 1975 Leslie and others highlighted that bovine tuberculin PPD¹ had both sensitivity and specificity advantages over human PPD and thus bovine tuberculin was used from 1976. In 1980 tuberculin potency issues resulted in change in the supplier. In 1994 an Irish reference preparation for bovine tuberculin PPD was calibrated against the International Standard and since then Ireland has used a standardized bovine tuberculin PPD at or about 30,000 I.U./ml PPD (+/-10%) and avian tuberculin 25,000 I.U./ml PPD (+/-10%) matched within 500 I.U./ml to ensure optimum specificity and uniform performance. Tuberculin potency is periodically validated in naturally infected tuberculous cattle against the Irish reference preparation. The Single Intra-dermal Comparative Tuberculin Test (SICTT) is the most specific tuberculin test available and the greater the potency of bovine tuberculin relative to the

1. Tuberculin PPD (bovine or avian) is a preparation obtained from the heat-treated products of growth and lysis of *Mycobacterium bovis* or *Mycobacterium avium* (as appropriate) capable of revealing a delayed hypersensitivity in an animal sensitised to microorganisms of the same species.

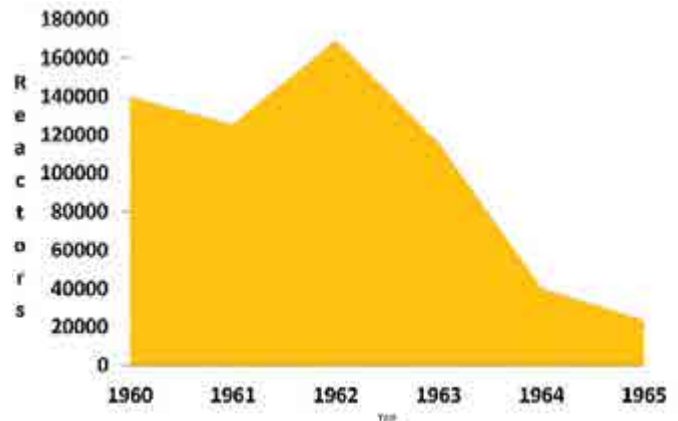


FIGURE 1: Bovines removed as reactors to the tuberculin test, 1960 to 1965.

In Ireland an initial pilot programme to assess levels of infection commenced in 1950. A voluntary bTB eradication programme followed in 1954 and in 1957 the compulsory programme started, on an area basis, extending nationwide by 1962. In October 1965 the Government of the day optimistically, based on the observed downward trend, (Figure 1) declared the country virtually free of tuberculosis.

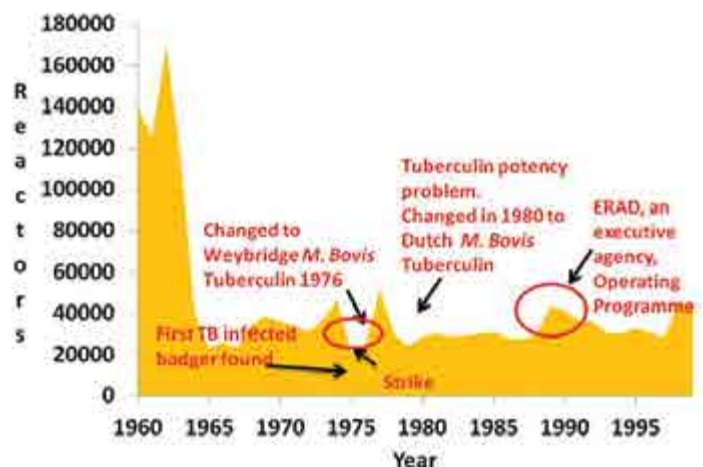


FIGURE 2: Bovines removed as reactors to the tuberculin test, 1960 to 1999 with key events depicted.

avian tuberculin the less the specificity and the greater the sensitivity of the SICTT and vice versa. It was only on accession to the EEC by UK, Ireland and Denmark that the SICTT was incorporated, by Directive 80/219/EEC, into the EU Trade Directive which originally had only allowed use of the Single Intradermal Tuberculin test (SIT).

The Irish bTB eradication programme, has evolved as the concept of understanding and managing risk developed and while the basic principles laid down in the 'trading' Directive, continue to be met, the programme has been enhanced by additional measures in an effort to achieve eradication. Multiple reviews and copious recommendations which the then current wisdom perceived as the reason for the 'stalled programme' led to the introduction of various other controls. Many of these controls, such as extended status withdrawal for infected herds have been later abandoned as not providing any significant benefit to bTB Eradication.

In 1988, ERAD, a specialised executive agency was established to implement a vigorous four-year eradication programme. The board, represented various interests, including farmers and veterinarians, involved in TB eradication. A strategic multi-annual plan was developed, a budget provided and an ambitious target set to reduce the reactor numbers by 50%. Herds/areas were categorised according to disease incidence, with a specific strategy applied to each category and there was a huge increase in the number of tests performed. However, as can be seen from Figure 2, despite an increased reactor identification and extraction rate throughout the four years of the programme, the reactor numbers thereafter stubbornly remained at the ~30,000/annum levels.

The Tuberculosis Investigation Unit, was established in 1989, based in the then Veterinary Faculty in University College Dublin. The function of the unit was to investigate the factors that militate against the eradication of tuberculosis in cattle at the national or regional levels, and to identify means of improving the rate of eradication. *In association with field and laboratory based operations, there has since been an extensive programme of research (much of it epidemiological) to address gaps in knowledge, to objectively evaluate alternative strategy options, and to critically assess the implementation of disease control strategies.* Although the role of its replacement the Centre for Veterinary Epidemiology and Risk Assessment (CVERA) has now broadened considerably, it continues to manage and analyse retrospective and concurrent data relating to the occurrence of tuberculosis in cattle and to undertake projects to answer specific questions and assess epidemiological elements, various components of infection and the role of wildlife; the elements of BTB diagnosis (Tuberculin skin test, Interferon- γ assay – introduced to Europe at the initial M.bovis conference held in Dublin in 1991, ELISA, anamnestic ELISA etc.) and developments therein. The effect of a recent tuberculin test and the nature of the response to tuberculin test have been elucidated, strain typing of M. bovis undertaken and the role of environmental mycobacteria investigated. A 'Singleton Protocol' was developed to provide a means for rapid derestriction of herds where TB is considered unlikely epidemiologically and there is no confirmation at laboratory level.

Until 1996, a pre-movement test was required, then abandoned as neither cost effective nor contributing to bTB eradication. There was also little evidence of onward transmission of infection from moved infected animals. Accordingly, with some herd/animal risk based exceptions, animals may move for up to 12-months from the date of their last SICTT. Breakdown severity, during a bovine tuberculosis episode, is a predictor for future herd breakdown as is contiguity to a herd undergoing a high-risk breakdown, defined as within herd acquisition and spread of TB (≥ 2 infected animals). Therefore, herds with a high risk history and their contiguous herds are tested more frequently and the window of opportunity for movement from such herd is curtailed. Inconclusive reactors, remain a risk for life and therefore notwithstanding that they may pass multiple SICTTs subsequently they are confined to their herd of disclosure for life.

Knowledge of disease epidemiology (including causation and, if infectious, transmission and maintenance of infection) is central to the development of control strategies. In 1958 Francis, speaking of the difficulties in final eradication of bTB recommended that to achieve complete success tuberculosis had to be dealt with in all species. In 1974 the first infected badger (*M. meles*) was detected in Ireland and by the mid 1980s infected badgers had been found throughout the whole country. Over the subsequent 30 years, the role of wildlife in bovine tuberculosis became clearer as the same strain types of *M. bovis* were detected in both badgers and cattle. Tuberculosis was described as a re-emerging disease at the interface of domestic animals and wildlife by Palmer who cautioned that it will not be possible to eradicate *M. bovis* from livestock until transmission between wildlife and domestic animals is halted, which will require collaboration between stakeholders. Some wildlife species, principally the badger in the United Kingdom and Ireland, the Australian possum in New Zealand (but not Australia), and water buffalo in Australia, have been recognised as significant reservoirs of *M. bovis* with endemic self-maintaining infection in these species constituting a major obstacle to disease control programmes. In Australia, elimination of wild water buffalo, and feral cattle from endemically infected areas was a major component of the eradication campaign in, now bTB-free, Australia. New Zealand similarly employed strict possum population control measures, and very considerable progress has been made. Michigan State in the USA had been TB-accredited-free from 1979, with no tuberculous cattle detected for 5 years. However, in 1994 a hunter found a tuberculous deer and it is accepted that endemically infected deer were spilling back infection to local cattle farms. Consequently on-farm risk mitigation measures against TB transmission from deer to cattle are recommended. TB has been reported in wildlife in a number of European countries struggling to eradicate tuberculosis in bovines. It is becoming increasingly obvious that wildlife populations with endemic TB pose a significant constraint to final TB eradication in cattle. In epidemiological terms, disease can persist in some wildlife species, creating disease reservoirs, if the basic reproduction rate (R_0) and critical community size (CCS) thresholds are achieved. Thus eradication efforts require elimination of *M. bovis* transmission between wildlife reservoirs and cattle. However, at present, the sympatric wildlife reservoir is the major impediment to the eradication of bTB in Great Britain, and Ireland and to ignore this impediment would be tantamount to dismissing one of the basic tenets of eradication.

DAFM commissioned a number of research projects to accurately estimate the contribution of the tuberculous badger population. The results showed a reduction in the number of reactor cattle with a significant difference between the removal and reference areas in each study area. Furthermore the herd breakdown risk was significantly lowered in the culled relative to the control areas for up to a decade after the end of the trial. Ireland and the U.K. are collaborating to develop a vaccination strategy for badgers in order to progress bTB eradication. BCG vaccine is protective in captive badgers. Field trials will elucidate the impact of vaccine in wild populations. Pending vaccine development, manufacture and licensing for general delivery to a wild population, Ireland operates a non-selective badger cull regime directed, since 2004, to areas with a chronic history of bTB. Culling reduces infected badger density and overall TB prevalence in badgers to minimize spill over to cattle. There has been an overall drop in mean TB prevalence in culled badgers from 26% in 2007 to 11% in 2011. The decline in infection levels in cattle is at least partly attributed to the culling programme. A recent simulation model also supports the hypothesis that culling strategies appeared to be the most effective method for TB control in badgers. A reduction in both badger population density and TB infected badgers followed by long term vaccination may be effective in reducing risk to cattle herds. Pending oral vaccine development culling is gradually being replaced by BCG injection with a non-inferiority study commenced in 2014 to determine if allowing a vaccinated badger population to rebuild in previously culled areas is viable in terms of levels of bTB in sympatric cattle.

TABLE 1: Cattle Population and Tuberculin testing statistics over four decades; five-yearly from 1960-1995, then yearly.

Year	Herds	No. of newly Restricted Herds	% Herd incidence	Cattle Population Tested	Number of Animal Tests	No. of Reactors	% Animal Disease Prevalence	APT **
1960	~250000			4,683,700	*	139,881	2.99	-
1965				5,359,300	*	23,378	0.44	-
1970				5,956,500	*	35,982	0.6	-
1980				6,908,900	8,878,924	29,827	0.43	3.6
1985				6,907,200	11,180,602	32,608	0.47	2.9
1990	172,765	13,489	7.8	6,899,929	12,427,146	41,419	0.60	3.3
1995	154,401	9,518	6.2	7,174,016	10,112,939	33,180	0.46	3.3
1996	149,128	8,867	5.9	7,412,933	10,073,859	30,400	0.41	3.0
1997	145,209	8,139	5.6	7,725,634	9,919,074	28,647	0.37	2.9
1998	142,302	10,055	7.1	7,946,989	10,677,291	44,498	0.56	4.2
1999	138,263	10,660	7.7	7,569,735	10,749,580	44,903	0.59	4.2
2000	133,542	10,785	8.1	7,032,407	10,304,162	39,847	0.57	3.9
2001	130,525	9,195	7.0	7,097,430	9,402,196	33,702	0.47	3.6
2002	127,711	8,338	6.5	7,025,096	9,400,065	28,930	0.41	3.1
2003	125,512	7,771	6.3	6,937,424	9,141,551	28,002	0.40	3.1
2004	124,410	6,788	5.9	6,991,988	8,824,533	22,967	0.33	2.6
2005	123,322	6,647	5.5	6,407,456	9,060,044	25,884	0.40	2.9
2006	122,392	6,386	5.4	6,260,133	9,000,519	24,173	0.39	2.7
2007	120,652	7,046	6.0	6,084,037	9,143,719	27,711	0.46	3.03
2008	118,030	6,837	5.9	6,150,163	9,211,058	29,901	0.49	3.25
2009	117,287	5,860	5.1	6,025,656	8,963,097	23,805	0.40	2.66
2010	116,815	5,304	4.7	5,751,564	8,390,750	20,211	0.35	2.41
2011	116,061	4,780	4.2	5,819,281	8,332,285	18,531	0.32	2.22
2012	115,787	4,856	4.3	6,145,469	8,534,677	18,476	0.30	2.16
2013	115,765	4,430	3.9	6,146,958	8,474,961	15,612	0.25	1.84
2014	114,508	4,111	3.6	6,115,528	8,445,262	16,145	0.26	1.91
2015	115,091	3,823	3.32	6,321,196	8,429,602	15,317	0.24	1.82

* Accurate figures for the total number of animal tests per year were not available until 1978.

** The APT is used as a measure of the incidence of disease compared to the level of testing being carried out. The APT figures represent the number of reactor animals disclosed per 1,000 tests.

*** The RPT is used as a measure of the incidence of disease compared to the total population of animals. The RPT figures represent the number of reactor animals disclosed per 1,000 animals.

The trend in cattle population and disease incidence since 1960 is presented in Table 1. In 1954, when the eradication programme commenced, there were some 250,000 herds with 4.5 million cattle registered in Ireland with an animal test reactor incidence of 17% (cows 22%, other cattle 8%). Over the course of the programme to date, in excess of 300M individual animal tuberculin tests have been conducted. Human zoonotic tuberculosis is now uncommon in Ireland.

In summary Ireland has as the basis of the modern and ever evolving bTB eradication programme:

- Mandatory herd (epidemiological unit) registration,
- Individual bovine animal unique identification,

- Individual animal passport
- A computerised Animal Identification and Movement system (AIM)
- An Animal Health Computer System (AHCS)

A comprehensive programme of disease surveillance including:

Farm-based testing:

- mandatory SICTT annual test of all herds, testing herds contiguous to or otherwise epidemiologically linked to infected herds, check-tests of at risk herds and herds in 'at-risk' areas, a herd test six-months following restoration of status, and
- Veterinary inspection of all bovine carcasses in abattoirs.

Figure 3: I

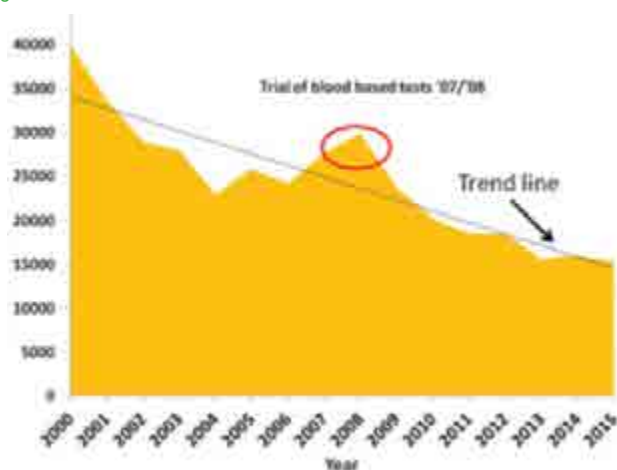


Figure 3: II

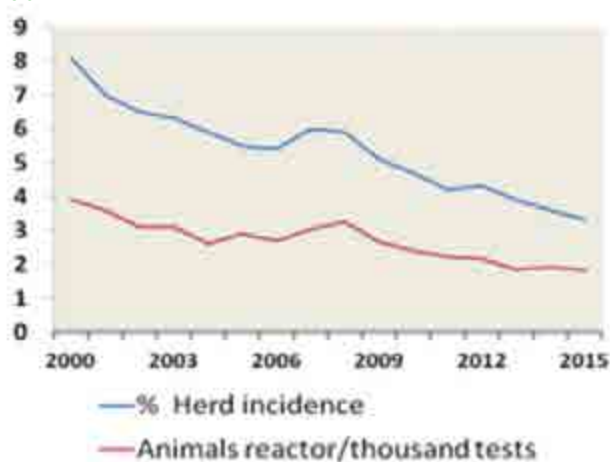


Figure 3: III

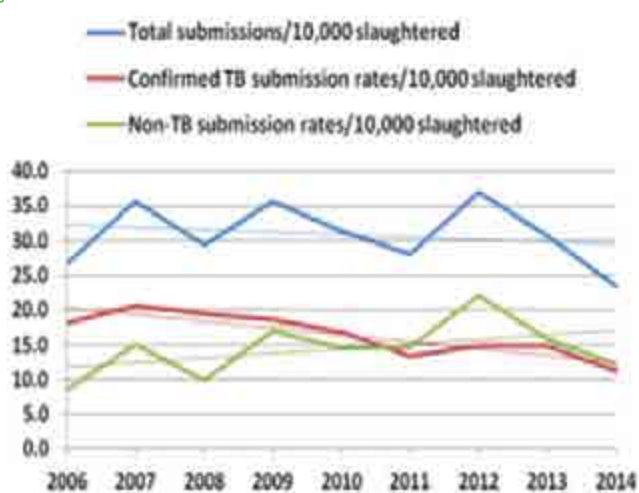


Figure 3: IV

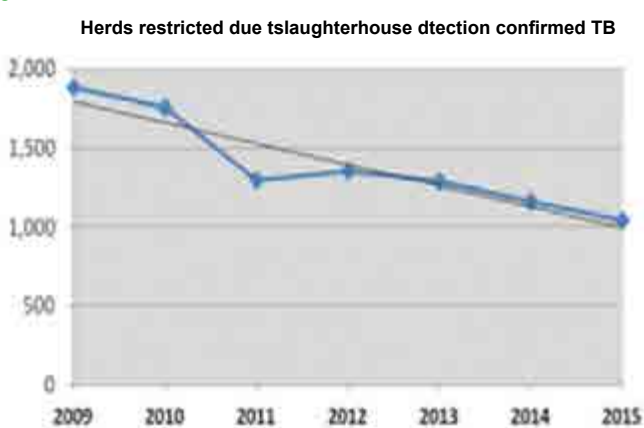


Figure 3: Key performance indicators

- I. Animals removed as reactors under the bTB eradication programme 2000-15 with trendline;
- II. Herd incidence and Animal reactor per 1000 animals tested SICTT (APT) 2000-15
- III. Slaughterhouse lesion submission rates, suspect and confirmed 2006-2014
- IV. Herds restricted due to slaughterhouse detection of confirmed bTB 2009-14 with trendline.

Disease controls

- severe interpretation of SICTT when infection established;
- Interferon-γ assay, ELISA and anamnestic ELISA use in problem herds;
- prompt removal of reactors;
- epidemiological investigations;
- outward movement limitation from H-breakdown herds post-status restoration
- SICTT at ~60-day intervals until two clear tests in succession;
- hygienic controls on infected holdings and vehicles;
- trace of (potentially) TB infected animals from and forward to other herds;
- herd depopulation where TB levels or duration indicates it is necessary to clear the herd and/or protect the neighbourhood;
- restriction of contiguous herds >4-months since previous test; and
- Confinement of inconclusive reactors to herd of disclosure for life.
- Badger population controls (culling), minimum 3-years followed by gradual replacement of cull areas by badger vaccination (BCG by injection)

Compensation

- market valuation of reactors but with a maximum allowable valuation.
- Income and hardship support payments

Quality control

- potency assay of tuberculin in naturally infected cattle
- SICTT conducted by specifically authorised Veterinarians;
- monitor on equipment, test performance and results for each testing Veterinarian;
- monitor on abattoir surveillance for TB suspect lesions
- computer monitor of testing performance,
- ‘Singleton Protocol’ monitor on reliability of SICTT and progress towards bTB eradication
- QC checks on testing facilities; and reactor animal examination including use of Interferon-γ assay.

The Interferon-γ assay is used as an adjunct to the tuberculin test, particular since 2012, so as to identify potential infected animals for removal. Since 2000 bTB levels, have been declining, herd incidence, number of reactors (notwithstanding increased removal of Interferon-γ assay positives) and number of detections of TB lesions at slaughter in non reactor cattle are all indicative that progress is being made towards bTB eradication. Figure 3

Conclusion

Full compliance with EU Directive 64/432/EEC, ensures that Irish farmers meet the conditions for trade within Europe and beyond. Recent progress is reflected in the education in bTB levels as measured by the key performance indicators. The consistent application of the programme ensures that bTB is no longer the scourge it was.

Eradiation of bTB is the ultimate objective of the national programme and considerable progress has been made over the last 15-years, sufficient to support the goal of bTB eradication in 2030. Realistically, however, this can only be achieved by simultaneously tackling the disease in all the maintenance hosts in which the disease is endemic and which share the same environment as bovine animals. As disease levels decline the controls where herds and animals are detected infected will perform become increasingly severe until eradication is finally achieved.

National Cattle Health Programmes- an Irish Perspective

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Introduction

The agri-food sector is Ireland's largest indigenous industry, contributing €26 billion in turnover and generating 12.7% of merchandise exports. The sector accounts for 8% of total employment, and makes a particularly significant contribution to employment in rural areas. Food and beverage exports increased to a record value of €10.4 billion in 2014, representing an increase of 4% on the previous year and a 45% increase since 2009.

Ireland has a national cattle herd of some 6.9 million head. The annual calf crop is approximately 2.2 million, with similar proportions born in dairy and beef herds. There are approximately 116,000 cattle herds, of which 17,000 are dairy enterprises, 61,000 are beef breeding (suckler) enterprises and the balance are categorised as beef (28,400) or "other" (9,500). Husbandry systems are largely based on production from grass and as a consequence there is a significant bias toward spring calving to match grass growth, with 83% of calves born in the first half of the year and 43% in February and March alone.

Cattle and milk products, together account for approximately 70% of agricultural output. Given Ireland's high degree of self-sufficiency in these products, it is the international, rather than the domestic, marketplace that is, in large measure, their final destination.

Reflecting the importance of the agri-food sector to the national economy, there have been two significant strategy documents developed in recent years to guide its development, with particular emphasis on the cattle sector. The first of these, Food Harvest 2020 (<http://www.agriculture.gov.ie/agri-foodindustry/foodharvest2020/>), established significant growth objectives for the sector in the period 2010 to 2020, including:

- Increasing the value of primary output of the agriculture, fisheries and forestry sector by €1.5 billion;
- Improving the value-added in the sector by €3 billion;
- Achieving an export targets of €12 billion for the sector
- Increasing the volume of milk production by 50%;
- Adding 40% to the value of the beef sector.

Building on the progress already made against these targets, the Department of Agriculture, Food and the Marine (DAFM) recently launched Foodwise 2025 (www.agriculture.gov.ie/foodwise2025) a national ten year strategy for the agri-food sector to 2025, setting new goals of 70% and 85% increases in the gross value-added of the sector and of the value of exports respectively relative to a 2012-baseline. Key actions in relation to further development of people, innovation, market development and competitiveness are identified with sector-specific recommendations that also address the need for environmental, social and economic sustainability.

Both of these documents recognise the key contribution which improved animal (cattle) health can make to achieving these goals.

Animal Health Ireland (AHI)

AHI (www.animalhealthireland.ie) was established in 2009 as an industry-led, not-for-profit, partnership between livestock producers, processors, animal health advisors and government to provide national leadership and co-ordination of animal health issues that are not regulated at national or European Union-level (More et al., 2011) but until recently

there has been no national coordination of non-regulatory animal health issues. This gap has recently been filled with the establishment of Animal Health Ireland (AHI).

Prior to this, public-private sector interaction in animal health policy in Ireland was typified by government-led and -funded programmes such as those for tuberculosis, brucellosis and bovine spongiform encephalopathy, reflecting the significant risks posed to public health and market access and consequently the predominance of public good arising from their control and eradication.

AHI's vision is to be recognised as a world-class resource enabling Irish farmers and the agri-food industry to achieve and maintain the highest international standards of animal health, to improve the profitability and sustainability of their enterprises and to enhance the value and competitiveness of Irish products in the marketplace. Given the importance of the sector, AHI has to date only addressed diseases of the cattle industry.

One of AHI's first tasks was to identify and prioritise the cattle health challenges facing this sector. This was addressed through a policy Delphi study with national experts and surveys of beef and dairy farmers to prioritise diseases and conditions based on cost (to farmers, to agribusiness), impact (on farmers and their animals), international perception and impediment to market access (More et al., 2010) national animal health services have been a government, rather than an industry, responsibility. In 2009, Animal Health Ireland (AHI). The top seven disease priorities included both infectious agents (infectious bovine rhinotracheitis [IBR], bovine viral diarrhoea [BVD], Johne's disease [JD]) and multifactorial conditions (infertility, udder health, lameness and diseases of young calves).

Based on this work, and taking into account both the probability of achieving eradication (or significant control) of each disease within reasonable timeframes and the resources and expertise available, AHI initially established and rolled out four prioritized programmes for BVD, somatic cell count (SCC; CellCheck programme), JD and IBR with these being incorporated as key activities in a three-year (2012-2014) strategic plan with defined strategic objectives and annual targets. These have been carried forward into a further three year (2015-2017) strategy document, supplemented with an additional programme to capture and use abattoir data on liver and lung lesions (Beef HealthCheck). In addition, activities are undertaken under four further work areas: parasite control, CalfCare, biosecurity and animal health economics, with outputs either supporting priority programmes or equipping farmers, vets and advisors to achieve change at individual farm level.

Reflecting the partnership ethos of the organisation, and the greater, albeit variable, private good that accrues from addressing these diseases, funding is provided by a range of private and public sector stakeholders, including dairy and beef processors, farmers' organisations and livestock marts, professional, advisory and support services and government and state agencies.

For each priority programme or work area, AHI has established a technical working group (TWG) of experts tasked with the development of detailed factual resources, development of tools to aid the control or eradication of the disease at farm level, development of policy options where appropriate for disease control/eradication and identification of areas for future research.

Where a decision has been taken to formalize a programme at national level, on either a voluntary (JD, Cellcheck) or a compulsory (BVD) basis, a cross-industry Implementation Group (IG) drawn from stakeholder organisations and chaired by AHI has also been convened to oversee their delivery. Consideration of the costs and benefits of a national approach, and allocation of these to different sectors including farmers, processors and government, has been identified as a key factor guiding decision making by IGs, and several studies have been carried out in this regard (Geary et al., 2013, 2012; Stott et al., 2012) cases treated, and

on-farm practices around mastitis management. The Moorepark Dairy Systems Model, which simulates dairying systems inside the farm gate, was used to carry out the analysis. The cost components of mastitis that affect farm profitability and that were included in the model were milk losses, culling, diagnostic testing, treatment, veterinary attention, discarded milk, and penalties. Farms were grouped by 5 BMSCC thresholds of $\leq 100,000$, $100,001-200,000$, $200,001-300,000$, $300,001-400,000$, and $> 400,000$ cells/mL. The $\leq 100,000$ cells/mL threshold was taken as the baseline and the other 4 thresholds were compared relative to this baseline. For a 40-ha farm, the analysis found that as BMSCC increased, milk receipts decreased from $\approx 148,843$ at a BMSCC $< 100,000$ cells/mL to $\approx 138,573$ at a BMSCC $> 400,000$ cells/mL. In addition, as BMSCC increased, livestock receipts increased by 17%, from $\approx 43,304$ at a BMSCC $< 100,000$ cells/mL to $\approx 50,519$ at a BMSCC $> 400,000$ cells/mL. This reflected the higher replacement rates as BMSCC increased and the associated cull cow value. Total farm receipts decreased from $\approx 192,147$ at the baseline ($< 100,000$ cells/mL). Social science studies are also increasingly used to gain a better understanding of factors that either prevent or motivate changes in behaviour in relation to the various programmes.

AHI PROGRAMMES

CellCheck

The CellCheck programme was originally developed in response to industry demand for a sustainable and coordinated approach to improving mastitis control. It was launched in 2010 and has focussed on building awareness, knowledge and capacity to facilitate improvements in mastitis control through the development of independent, science-based resources and training for both service providers and farmers. These have facilitated engagement between service providers, and the development of regional networks. CellCheck also enhances the regional support network and the consistency and quality of information available to farmers in relation to mastitis control. There has also been an increasing emphasis on working with industry partners to ensure that suppliers receive clear, consistent signals about the desired quality of raw milk produced in Ireland. CellCheck recognises that enabling the industry to work together to deliver consistent information and mastitis control solutions is the most sustainable model. It is also important for milk suppliers to recognise the role that all disciplines can play in improving mastitis control. Based on the premise that mastitis is a multifactorial problem, and therefore best addressed by a multidisciplinary approach, CellCheck is working to develop the capacity of the various service provider groups – vets, farm advisers, dairy co-op milk quality advisers and milking machine technicians – to work collectively to provide solutions and support for dairy farmers.

The establishment of a national SCC database, which allows trends in the national herd to be examined, and the impact of the programme to be evaluated, has been another key achievement of the programme.

The current goal set by industry is that 75% of the milk supplied by Irish farmers will have an SCC of 200,000 cells/mL or less by 2020.

Significant progress has already been made with data from milk recording herds showing that in 2015, 61% of herds had an annual average SCC of $< 200,000$ cells/mL, compared to 26% of herds in 2010. Bulk tank data from the national SCC database shows a similar trend, with the proportion of herds and milk volume with an annual average SCC $< 200,000$ cells/mL increasing from 39% to 45%, and 46% to 55% respectively, between 2013 and 2014.

Johne's Disease

Following a review of control programmes elsewhere (Geraghty et al., 2014) control activities and monitoring components of programmes in Australia, Canada, Denmark, the Netherlands, the United Kingdom

and the United States of America were individually reviewed. Factual accuracy of each review was checked by individuals involved in the respective programmes before the reviews were condensed and combined into a single document presented here, with the complete reviews of each programme available as supplementary material. There was considerable heterogeneity in key aspects of control activity design including goals, responses to declining participation, herd classification, recommended control measures and associated test requirements. The data presented will be of interest to organisations that are involved in developing new or existing regionally coordinated BJD control activities. (Geraghty et al., 2014, the JD TWG developed the technical details of a dairy Johne's disease control programme (JDCP). Following consideration of these by the JD IG, a pilot programme was initiated in 2013 with the following objectives:

To test, evaluate and refine the various programme components that would be required to support a future, extended JDCP with the following goals:

Bio-exclusion

To identify herds that test negative for JD and provide them with the knowledge and professional support to allow them to increase their confidence over time of being free of infection and to protect their herds from the on-going risk of introduction of this disease.

Bio-containment

To provide infected herds, or those having a low confidence of freedom from infection, with the knowledge and professional supports to allow them to control and reduce the prevalence of the disease over time and ultimately to achieve a high confidence of freedom from infection (bio-containment).

Market reassurance

To underpin the quality of Irish dairy and beef produce in the international marketplace.

To generate information on the control of JD on Irish dairy farms, including that relating to the economics of the disease and its control, to assist the design of a future, extended JDCP.

Key components of the pilot programme include herd screening of eligible animals, limitations on the sale of JD-positive/inconclusive animals and an annual on-farm veterinary risk assessment and management planning visit conducted by an approved veterinary practitioner.

The JD TWG has developed a system of herd categorisation, based on test results and movement data, which provides a measure of the degree of confidence that any given herd is truly free of infection at a particular point in time and a framework by which to evaluate progress in controlling the disease at individual herd and aggregate level (More et al., 2013) and separate sub-programs are followed for test-positive and test-negative herds. However, a test-negative herd result does not necessarily equate to JD freedom for reasons relating to disease pathogenesis and available diagnostic tests. Thus, in several countries, JD control programs define test-negative herds as having a "low risk" of infection below a specified prevalence. However, the approach is qualitative, and little quantitative work is available on herd-level estimates of probability of freedom in test-negative herds. This paper examines the effect over time of alternative testing strategies and bio-exclusion practices on JD risk in test-negative herds. A simulation model was developed in the programming language R. Key model inputs included sensitivity and specificity estimates for 3 individual animal diagnostic tests (serum ELISA, milk ELISA, and fecal culture).

A review of the pilot programme will be carried out in 2016 to determine the next steps in terms of its extension or expansion.

IBR

The programme aims to eradicate IBR from the national herd, subject to a positive cost-benefit analysis (CBA) and a mandate from AHI stakeholders. Work is ongoing on the CBA, including modelling of options for a national programme. The CBA will then be presented to AHI stakeholders and a decision taken on whether or not to progress to a national eradication programme, with a target date of commencement by 2019.

Beef HealthCheck

This is a new programme which is being developed by AHI in conjunction with industry partners and DAFM. The objectives of the programme are firstly to develop tools to assist farmers and their veterinary practitioners to control losses due to liver fluke and pneumonia through capture, analysis and reporting of abattoir data and secondly to provide data that will contribute to the development economic breeding indices that address incorporating health and disease. Health information is captured by veterinary inspectors using touch-screen technology during the public health inspection process in slaughter premises. This information is provided directly to farmers in batch-level reports and is also transferred to a central programme database (www.icbf.com) which is currently being developed to provide farmers and their veterinary practitioners with tools to assist them with herd health management as well as providing national-level data for monitoring and evaluation, including the losses associated with these conditions, improved fluke forecasting and genetic analyses.

BVD

Prior to initiating a national eradication programme, a review of other European programmes was undertaken (Barrett et al., 2011), followed by a national consultation and a cost-benefit analysis (Stott et al., 2012), before a decision to proceed was taken. A range of factors relating to the industry (herd size, type, calving profile, herd contacts, level of movements), logistics (sample collection, transport, laboratory capacity, availability of a national database), socio-economics (costs and benefits at farm and national level, farmer awareness and support), disease (prevalence) and political factors (support of government, farming and veterinary organisations) were also taken into account in deciding to proceed with the programme and on its structure and implementation.

The programme began with a voluntary year in 2012 (Graham et al., 2014) during which approximately 600,000 of the 2.1 million calves born were tested. This provided an opportunity to test the programme, to learn from farmers' experiences (Devitt et al., 2014) and for industry to demonstrate commitment to the programme. As a result, the BVDIG requested government to introduce legislation to enable a compulsory programme from 1st January 2013.

All herd owners must collect and submit ear tissue samples (collected using the official identity tag or a supplementary tag) to a designated laboratory, from where they are transferred on a daily basis to a central database (www.icbf.com), which then issues results to farmers by SMS message. Results are also shared with government databases, preventing movement of animals without a negative result.

2013 provided baseline national data, with 0.67% of calves born considered to be persistently infected (PI) and 11.3% of herds having positive results. In 2014 and 2015 these levels fell to 0.46%/7.6% and respectively, and 0.33%/5.9% in 2015. Further significant progress has been made in 2016, with only 0.14% of 700,000 calves born in January and February considered to be PI.

While immediate removal of PIs has been encouraged, their retention by some farmers in the absence of compulsory removal and compensation at notional market value has been an ongoing problem. Several studies and modelling work have (Graham et al., 2015a; Graham et al., 2015b), reinforced the importance of prompt culling. A number of measures,

including the provision of limited financial supports, the exclusion from other government-funded agriculture programmes of herds that retain PIs, the imposition of movement restrictions, the notification of neighbouring herds and investigation of herd breakdowns funded under the Rural Development Plan have all contributed to an improvement in the rate of removal.

After three years of testing, more than 98% of animals in breeding herds now have a known (negative) status, acquired either directly based on their having been tested within the programme or as indirectly through their having produced one or more negative calves. Based on these results, more than 54,000 breeding herds (%) have acquired a negative herd status.

Conclusions

AHI has made significant progress in developing national cattle health programmes since being established in 2009 which have made a significant contribution to the delivery of specific objectives in Food Harvest 2020 and Foodwise 2025. The achievements to date owe much to active collaboration amongst key stakeholders, guided and led by AHI, and supported by a multi-stranded, comprehensive communications strategy. The AHI model shows the potential and challenges for industry-led programmes built on consensus to deliver real progress.

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Predictive Biology; The Future for Mastitis Control?

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An ultimate goal of disease control programmes is to accurately predict future events such that measures to prevent disease can be identified in advance. In this paper we consider the current context of mastitis control on dairy farms and discuss future possibilities where predictive biology may provide insights into the prediction and prevention of disease.

Context of mastitis control

Mastitis remains an important condition of dairy cattle on a global scale. As well as causing substantial financial losses (Halasa et al., 2007), changes to the quality and storage characteristics of milk (Barbano et al., 2006) and a negative impact on cow welfare (Milne et al., 2003), with the increasing concerns over the sustainability of farming and global food security, the environmental impact, wastage and inefficiencies associated with mastitis have come sharply into focus (Hospido and Sonesson, 2005). In the UK, for example, the milk retrieved if half of all clinical mastitis cases could be prevented would be sufficient to supply 1.5 million UK consumers per year. Furthermore, mastitis is one of the most common reasons for use of antimicrobials in dairy cows. With current concerns regarding the emergence of antimicrobial resistance in agriculture, there is a widespread drive to reduce use of antimicrobials in food producing animals.

Brief history and current approaches to mastitis control

Generic approaches to mastitis control have been discussed and implemented for many decades. At the start of the 19th century, mastitis had been identified as an important condition and the pathogens associated with mastitis were being worked out (Henderson et al., 1904). By 1918, sources of infection and routes of transmission were being considered in the hope that infections could be reduced (Jones, 1918). Antibacterial treatments for mastitis were used in the 1930s (Stevenson, 1939) and it was already noted in 1963 that mastitis was the most common reason for the use of antibiotics in cattle (Uvarov, 1963). By the early 1960s, information on the spread of contagious mastitis was being defined and five major elements of mastitis control were put together as a 'Five-Point Plan' (Neave et al., 1966).

Whilst we have moved on from this era to some extent, it is perhaps surprising that there are not more high-tech solutions for mastitis control. Whilst parlour automation has increased and improved, and in many developed dairy nations, classical contagious mastitis pathogens are now at low prevalence, there remains a reliance on generic control strategies that originated in the 1960s. In terms of barriers to implementing mastitis control on farm, there has been recognition that understanding human behaviour including how to 'change the minds and actions' of people involved with mastitis control is crucial (Jansen et al., 2010, Higgins et al., 2012, Jansen and Lam, 2012).

In general, approaches to providing advice on mastitis control might be divided into two types; 'reactive' and 'prospective'. Reactive mastitis management reflects a veterinary practitioner, advisor or referral service being asked to assess a problem situation, whether long standing or a sudden outbreak. Such investigations often include analysis of farm records and current management. Samples may be taken to assess pathogen involvement and may include sub-species (strain) typing to pinpoint bacterial sources (Munoz et al., 2007) A prospective approach

to mastitis control incorporates many of the principles of herd health management; setting of goals, on-going monitoring of disease and updating of management practices when goals are not achieved (Green et al., 2012).

The problem with both reactive and prospective approaches to mastitis control is that they rely on current farm information to define what is happening and therefore what to change. In this sense, they are both in fact 'reactive'. To transform mastitis control, an ideal situation would be to make use of current data to predict what is likely to happen in the future, and to predict different farm outcomes that would be expected in light of different decisions; such an approach could be termed 'predictive' mastitis control.

With advances in on-farm technologies, scientific approaches and data handling capabilities, opportunities in this field are becoming reality. We give four examples below in which research into biological processes aims to make predictions about mastitis and its control.

Predictive biology; examples in mastitis control

1. Predicting cow susceptibility to mastitis

Predicting the susceptibility of a cow to intramammary infection can be of use in the management of mastitis, not only in terms of breeding decisions, but also to allow individualised management practices. To some extent, disease susceptibility will be dictated by genetic influences and 'genomics', the prediction of phenotype from genomic markers, is possibly the field in which prediction of performance is most advanced. Although understanding genetic determinants of animal disease is at a relatively early stage, rapid advances are occurring in this area and there should be opportunities to improve the understanding of cow resistance to mastitis, as long as high quality phenotypic information is available. For mastitis, it is unlikely that one or a few genes will be responsible for most of the variation in cow mastitis resistance, and thus a truly polygenic approach will be necessary. It seems likely from studies to date that an integrated polygenic approach to genome investigation is likely to be beneficial in explaining the overall impact of genetic variability; similar studies have proved invaluable in deciphering the genetic involvement for human diseases (Lin, 2015, Musliner et al., 2015). Susceptibility to mastitis, however, is unlikely to be solely dependent on an animal's initial genetic make-up. Environmental influences alter susceptibility, partially through epigenetic changes and partly through direct physiological modifications. Evaluation of how the environment interacts with the cow's expressed genome may allow bespoke, individual management of animals.

An example of a direct modification of disease susceptibility for mastitis is the microbial community (microbiome) in the mammary gland. It is increasingly clear that the health and well-being of mammals is related to their colonising microbial communities (Ley et al., 2008). Some microbiomes might offer protection from infection by occupying niches that could otherwise be occupied by pathogens or by priming the immune system to respond to infection (Kau et al., 2011). The risk of disease may be influenced by the mammary microbiome and understanding this community could provide a new angle on the control of mastitis (Hunt et al., 2011). In a 2012 paper on the microbiome of the bovine mammary gland, differences were identified between the microbiomes of healthy and infected quarters, indicating microbiomes that varied by disease state (Oikonomou et al., 2012) An understanding of the mammary microbiome may enable temporal predictions to be made about individual or group resilience to mastitis and facilitate manipulation of this community to optimise mammary gland health. Research in this area is on-going.

2. Pathogen phenotypes; sub species predictions

The pathogens that cause mastitis in dairy cows are generally recognised and considered at species level in terms of their phenotypic characteristics on farm. For example, phenotypic traits include being

associated with 'contagious' or 'environmental' transmission, chronic or acute disease, long or short duration rise in somatic cell count (SCC) or the carriage of specific antimicrobial resistance (AMR) genes. However, it is clear that such characteristics will be different for different sub species within the major species of mastitis pathogens and therefore linking sub species to phenotypic infection patterns will help in terms of making predictions on farm.

For strain typing to be effective as a predictive technology, fast throughput systems, portable between laboratories will be needed that can accurately differentiate clinically important strain phenotypes. An example of such a system is Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). MALDI-TOF-MS produces a protein 'fingerprint' of ribosomal proteins quantified as the amount of each protein present, defined by its molecular weight. MALDI-TOF-MS is routinely used in many microbiological laboratories for species determination of bacteria and fungi, and can also be used for subtyping of bacteria. It has also been used successfully to differentiate bacterial sub species according to phenotype (Moura et al., 2008, Nagy et al., 2013).

We have recently conducted projects using MALDI-TOF-MS and multilocus sequence typing (Davies et al., 2016) to attempt to differentiate phenotypes of *S. uberis* putatively associated with either apparently 'contagious' or 'environmental' transmission. Results will be presented.

3. Predicting cost effectiveness of management changes

Since resources will be limited on a dairy unit, when assessing competing disease control strategies, it would be valuable if interventions could be evaluated and prioritised not only according to probable efficacy, but also to likely return on investment. The effective use of farm resources requires an understanding and weighing up of the probable outcomes of implementing one intervention rather than another. This is a dilemma faced by farm advisors and decision makers and it would be useful when deciding whether to employ resources in one area, to be able to compare, for example, the probability of a net benefit in that area with all other potential areas where resources could be employed (Briggs and Gray, 1999). This idealised approach is difficult to achieve in terms of mastitis control because data from intervention studies to assess the efficacy of different control policies is sparse. In reality it is of limited use to estimate some 'average' cost benefit of an intervention, rather we would like to know the probability of achieving different levels of cost benefit. An approach to probabilistic cost-effectiveness analysis adopted in the human medical field is a Bayesian method termed comprehensive decision modelling (Cooper et al., 2004, Ades et al., 2006a and b, Griffin et al., 2008). An example of this approach, applied to the cost effectiveness of mastitis control interventions, will be described.

4. Predictions from simulation

Our final example uses the technique of data simulation - this is a method to combine many sources of information to evaluate many different 'what if' scenarios. Simulation-based techniques can be particularly useful for the evaluation of hundreds of thousands of theoretical sets of farm circumstances when obtaining the equivalent experimental information would be impractical and expensive. It is also of considerable value to assess the impact of uncertainty in research findings, a critical area often overlooked. Simulation based studies use parameters derived from 'real life' (where possible) and combines these in ways to make onwards predictions. The goal is to identify patterns in the simulated scenarios to identify circumstances when specific control measures are likely (or not) to be useful and to rank the measures in order of usefulness. A major advantage of simulation studies is that parameters for the simulation can encompass a wide variety of sources such as previous research or expert opinion, and synthesise these to produce insights not previously captured.

During the conference, we will provide two recent examples of simulation

studies that have been used to improve the understanding and make predictions about mastitis. In the first, the impact of mastitis on herd reproductive performance was evaluated (Hudson et al., 2015) and in the second, the cost effectiveness of mastitis treatment was assessed with and without the use of an 'on farm culture' (OFC) technique (Down et al. submitted).

Conclusions; Big data – into the future...

The quantity of data and technology available on-farm will continue growing rapidly; opportunities will exist to harness these to make useful biological predictions. For instance, can we integrate information on cow susceptibility, pathogen behaviour and farm interventions to simulate different choices and offer 'real time decision support'? Essentially this would mean identifying the best decision (e.g. management change) in a given set of circumstances and its probability of success. The key will be to ensure results are clear, relevant and accessible to the end user. Is this the future for mastitis control?

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Economics for the veterinary practitioner: From burden to blessing

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Introduction

Some endemic diseases are implicitly associated with dairy production. These, so called, production diseases do cause large negative economic effects. Because of the chronic nature of production diseases, economic damage is spread out over the year, and a large part of the economic damage, such as lower milk production are often unnoticed by the dairy farmer. Farm accounting reports give all kinds of detail about the costs of production but these are in terms of feeding costs, machinery costs, and costs for animal improvement. The factor health costs only comprise costs for drugs and the veterinarian, which is only a small proportion of the total economic damage of a production disease. Still economic arguments are often used by farmers not to follow an advice or even to complain about the costs of veterinary service. Without good knowledge about the economics of animal health, these economic arguments are often seen as a burden for the veterinary practitioner. Instead of following good advice, farmers often “just” neglect that advice because they say prevention is too expensive.

A good understanding of the costs of a disease is important to support decisions of farmers with regard to animal health. It is important that this understanding goes beyond averages that are provided in literature. All calculations of costs of disease and cost-effectiveness of preventive and curative measures can be regarded as averages for a certain situation. Costs of diseases vary from farm to farm. This is not only dependent on the incidence of disease but also on the effects of disease on production, culling and mortality and price levels (Halasa et al., 2007). In order to support decisions of farmers, the advisor must be able to interpret such published data to translate them to the specific situation of an individual farm. Therefore, insights in the theories behind economic calculations in the field of animal diseases is necessary. In this contribution, an economic decision making framework will be described.

ANIMAL HEALTH FROM AN ECONOMIC PERSPECTIVE

Factors that Determine the Costs of Disease

The cost factors making up the total costs of disease consist of two big groups: failure costs and preventive costs (Hogeveen et al., 2010). Failure costs are the costs that are associated with the occurrence of the disease. The most obvious failure cost factors are those associated with treatment. Costs for diagnostics, veterinarian and drugs consist of expenditures and are very visible. Extra labour to treat diseased animals and discarded milk and meat are also visible, but it can be more difficult to establish the monetary value of these factors. The second group of failure cost factors are associated with production losses. Production diseases can lead to a decreased milk yield, lagging growth, a decreased product quality, increased mortality and an increased risk of culling. At the cow level, there are trade-offs between treatment and production losses. Better treatment will decrease the production losses. This means that the level of treatment can be optimized (e.g., Down et al., 2013; McArt et al., 2014; Steeneveld et al., 2011). Finally, a high incidence rate of a disease can increase the risk of new cases of disease, either because of infectiousness of diseases (one infected animal can infect another) or because of a reduced immunity due to disease. For instance, in the case of hyperketonaemia, the observed production losses can mainly be attributed to other, following, diseases rather than to the case of hyperketonaemia itself.

The second group of cost factors are associated with prevention. These preventive costs include all preventive measures that are undertaken by the farmer to prevent occurrence of the disease. There is a wide range of possible preventive measures against production diseases. All farmers have implemented more or less preventive measures. These can be structural measures and are associated with the design of the barn and the milking parlour (measures that cannot be changed too easily). Most preventive measures are, however, part of the daily management of a dairy herd. Preventive costs consist of costs of diagnostics, labour costs, veterinary service (e.g., veterinary herd health and management programs), investments and consumables. Moreover, there exists a trade-off between the preventive costs and failure costs. More preventive action will lower the incidence rate of disease and vice versa. More on this relation will be provided later in this chapter.

Although the economic effects of production diseases differ between diseases, farmers and farming systems, and that the value of the cost factors differs between farms and farm systems, the economic principles behind them are the same. In the following sections, failure costs and preventive costs of different production diseases are described in more detail.

Failure Costs of Production Diseases

In an overview paper, Hogeveen and van der Voort (2016) summarized the failure costs of production diseases. Costs of clinical mastitis varied from € 87 to € 1,006 per case, with most estimates being in a range of € 200 - € 400 per case. The differences were due to differences in country, causing pathogens, breed of cattle and production levels. Overall, failure costs of mastitis were estimated to be € 71 and € 108 per cow on the farm per year. In a recent study, the total costs of mastitis were estimated on 109 Dutch dairy farms (data not yet published). On average, half of the total costs of mastitis (€ 240; failure costs plus preventive costs) consisted of preventive costs. However, there is a large variation between farms (Figure 1).

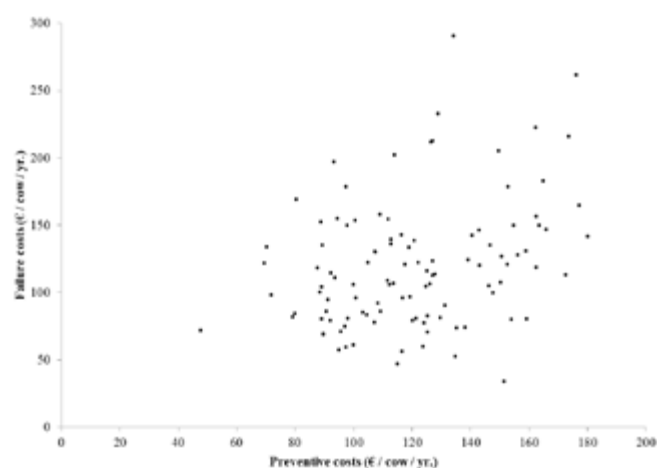


Figure 1. Estimated average preventive and failure costs associated with mastitis (€ per lactating dairy cow per year) per herd. Each point represents an individual dairy farm (n=108).

For lameness, costs per case were estimated to vary between € 111 and € 198 per case, depending on the cause and € 39 and € 124 per cow on the farm.

Recently two papers were published about the costs of subclinical ketosis (hyperketonaemia), leading to estimates of € 257 and € 265 per case. Current estimates of the costs of ketosis are only scarcely available in scientific literature. Costs of ketosis were estimated to vary from US\$78 to US\$289 per case of ketosis.

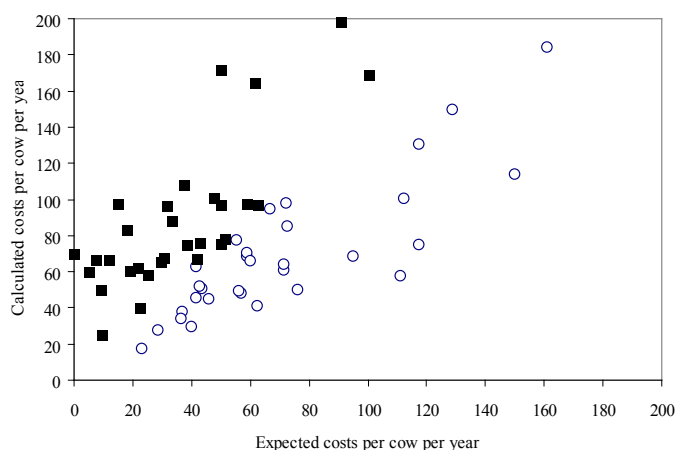


Figure 2. Expected economic losses per cow per year vs the calculated economic losses per cow per year (group 1: "good estimators" (○), and group 2: "under estimators" (■) (Huijps et al., 2008).

Even though there are quite some differences between the estimates, these estimates remain averages for the specific situation (often a standard farm for the region of interest) that was studied. In reality, there are large differences between farms. A study by Huijps et al. (2008) calculated the failure costs of mastitis for individual farms using a straightforward calculation tool. There was a large variation in farm-specific costs of mastitis, from € 17 to € 198 per cow per year (Figure 2). Moreover, before these calculations were made, the farmer gave his own estimate of the costs of mastitis. It appeared that only 8 % of the farmers estimated the failure costs of mastitis correctly. More than half of the farmers underestimated the costs of mastitis with more than 25%.

Since a good estimate of the failure costs of production diseases is a basis for economically sound decision making about preventive measures, it is important that farmers and their advisors do spend time on a proper estimation of the failure costs of production diseases. Quite some tools have been developed to make farm-specific estimations of failure costs of production diseases. Many of these tools are, as part of the extension task of universities, available on-line.

Economic Optimization of Preventive Measures

An economic approach in the selection of preventive measures can be done by looking at the possible preventive measures against production diseases and compare the costs of these preventive measures with the avoided failure costs when they are applied (Hogeveen and van der Voort, 2016). A preventive measure where the avoided failure costs are larger than the additional preventive costs has a positive net-benefit and is thus cost-effective. In such an analysis the following steps have to be taken:

1. Estimate the failure costs of the disease on this farm
2. Define a possible additional preventive measure or strategy that is applicable for this farm's situation
3. Estimate the effect of the preventive measure (e.g., in terms of reduction of incidence of disease)
4. Calculate the costs of implementation of the preventive measure
5. Recalculate the failure costs of the diseases with the expected reduced incidence on this farm
6. Determine the difference between the recalculated failure costs and the initial failure costs. This difference represents the expected benefit of implementation of the preventive measure
7. Compare the additional costs with the expected benefit to evaluate the cost effectivity.

Steps 2-7 may be repeated for additional preventive measures and may include multiple preventive measures (i.e. a disease prevention strategy).

Tool

Within in the EU IMPRO project (www.impro-dairy.eu) a cost-benefit tool has been constructed to support farmers and their advisors to support decisions on preventive measures against production diseases. Basically, the model estimates the total costs of the four common production disorders (Berge and Vertenten, 2014) major management systems, and fresh cow clinical conditions associated with ketosis in western European dairy herds. A total of 131 dairies were enrolled in Germany, France, Italy, the Netherlands, and the United Kingdom during 2011 to 2012. A milk-based test for ketones (Keto-Test; Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya, Japan; distributed by Elanco Animal Health, Antwerp, Belgium: mastitis, lameness, ketosis and metritis). In the cost-benefit module, total costs are defined as the sum of failure costs and preventive costs. It is possible to focus on one disease only or to estimate the costs of several diseases. A note should be made that the model estimates the costs of each disease individually and potential interactions between these diseases are not estimated.

The basic model input for the cost-benefit tool includes:

- Herd characteristics
- Milk production, number of dairy cows
- Herd health characteristics
- Mastitis, lameness, ketosis and metritis
- Price input
- Milk price, feed price, wage, replacement value, slaughter price, penalties, bonuses
- Farm characteristics
- Replacement rate, culling, death

With regard to herd health characteristics, different input is required for each specific disease. Mastitis requires input on the incidence of clinical mastitis and somatic cell count (SCC) classes, in which the first is derived from health recordings (or alternatively farmers' estimates) and the latter is derived from milk recordings. Lameness requires input on lameness scoring, ideally hoof trimming records are used. Alternatively users could estimate the incidence of moderately lame and severely lame animals in which it should be kept in mind that farmers tend to underestimate the severity of lameness (Brujinis et al., 2010). Ketosis requires input on the fat-protein ration, if fat-protein ration has been larger than 1.5 within the first 100 days in milk the cow is diagnosed with ketosis (Duffield et al., 1997; Krogh et al., 2011). Metritis has been divided into clinical metritis and subclinical metritis, diagnosis of these illnesses has to be performed by the farmer according to predefined definitions. Clinical metritis is defined as: an animal that is not systematically ill, but has an abnormally enlarged uterus and a purulent discharge detectable in the vagina, within 21 days post-partum. Clinical endometritis is defined as: the presence of purulent (>50% pus) uterine discharge detectable in the vagina 21 days or more after parturition, or mucopurulent (approximately 50% pus, 50% mucus) discharge detectable in the vagina after 26 days post-partum (Sheldon et al., 2006) and pathogenic bacteria often persist, causing uterine disease, a key cause of infertility in cattle. However, the definition or characterization of uterine disease frequently lacks precision or varies among research groups. The aim of the present paper was to provide clear clinical definitions of uterine disease that researchers could adopt. Puerperal metritis should be defined as an animal with an abnormally enlarged uterus and a fetid watery red-brown uterine discharge, associated with signs of systemic illness (decreased milk yield, dullness or other signs of toxemia).

Specific price input is requested from the user, however if a user is not sure about his input, default, country specific, values can be used. The most influential price input are requested: milk price, feed price, labour costs, replacement value of a dairy cow, costs of destruction, slaughter price, penalties paid and bonuses received as a consequence of an elevated/ low SCC.

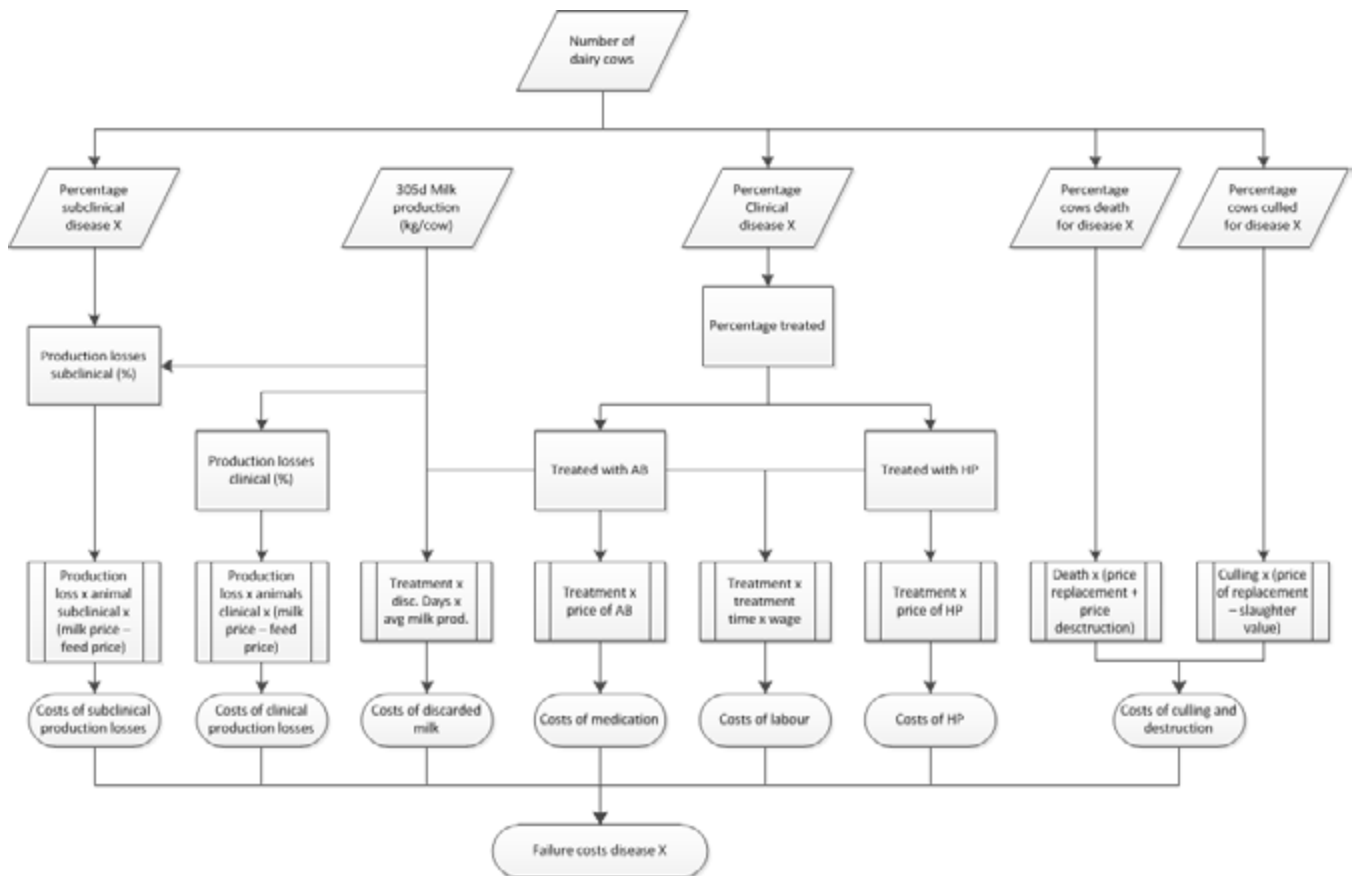


Figure 3 General structure of the calculation of failure costs. Input is requested at the parallelogram, technical assumptions are made in the squares and economic assumptions and calculations are made at the squares within a square, partial costs are derived at the pill shaped boxes, each path should be followed to estimate the total failure costs of a disease.

When all input data has been collected the users have to decide which preventive strategy they would like to implement on the farm. If these desired measure(s) is/are not included it/they can be manually added to the set of measures (up to 3 measures). Given the selected measures veterinary advisors/ farmers have to decide what the impact of the measures will be on animal health characteristics of the herd. E.g. if measure x is implemented on the farm by how much will it reduce disease y. The cost-benefit tool does not give any reference values of the expected effect of the chosen management measures. If the farmer is the user, it is advised he/ she consults the veterinary advisor on this topic.

As a first step, failure costs are calculated based on the general structure given in Figure 3. Failure costs generally consist of costs of (sub)clinical production losses, discarded milk, medication, labour, treatment, culling and destruction. Next, for each disorder at least 9 measures were derived from literature and/or expert knowledge and costs were estimated. The costs of each of these management measures consist of three distinct cost factors:

- **Labour**, costs of labour associated with performing the measure.
- **Consumables**, costs of material necessary to complete the measure
- **Investments**, costs of investments that are generally present on the farm for longer than one year

Using these factors default costs for each of these measures are calculated. Also expected effects of the preventive measures are built in the tool. Given the expected effect the model estimates current failure costs, potential reduced failure costs (after implementation of the selected measure) and potential increased preventive costs (after implementation of the selected measure). The difference between the current failure costs and potential total costs (reduced failure costs plus increased preventive costs) is the net effect of the management measures on farm income.

Besides the provided preventive measures, the user can define new preventive measures. Moreover, the user can change all assumptions that have been made and can thus adjust cost factors and effectivity of preventive measures to reflect the specific farm situation as good as possible.

Conclusions and Implications

On a dairy farm, production diseases are responsible for a considerable proportion of the costs of milk production. These costs consist of failure costs and prevention costs. The failure costs of production diseases are only for a part visible to the dairy farmer. Farmers tend to underestimate the failure costs of production diseases. Farm-specific calculation of the costs of production diseases is therefore necessary as a starting point to discuss potential preventive measures.

Because of the large variation in failure costs of production diseases between farms, there is room for investment in preventive measures. The net benefits of preventive measures should be calculated for a farm specific situation. A tool has been described and will be available on-line that can be used to calculate the net effect of a wide range of potential preventive measures.

Good knowledge of the economics of production diseases including ways to support farmers with economically founded advises to prevent production diseases may be a key to turn economics from a burden towards a blessing for the veterinary practitioner.

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Natural Treatments for Mastitis and Infertility

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Background

Since 2002, certified organic animals in the United States that are given antibiotics or hormones must be permanently removed from the organic sector (7CFR205.238c7)¹. Many skeptics believe that this reduces animal welfare. However, it cannot be stated strongly enough that the use of antibiotics on their own does not constitute animal welfare. Animal welfare is much broader – it includes how the animals experience life on the farm. In relation to reduction of antibiotic use, there is reason to believe that the United States certified organic livestock sector is leading the way globally in developing alternatives to antibiotic use and reducing antibiotic use in animal agriculture in general.

Over the course of the last 15 years, there have been epidemiologic studies that compare conventional herds to organic herds. The author was involved with two of studies that compared standard production and reproduction parameters which were conducted among dairy cattle herds in the Lancaster, Pennsylvania region using standardized data from Dairy Herd Improvement Association.

In 2006, data was analyzed from more than 30 herds in Lancaster, PA. The herds were matched for breed, herd size and located within one mile from each other. (Table 1 and Table 2)

Similar results were found in study conducted in 2016 with more than 60 herds in Lancaster, PA. The herds were once again matched for breed and herd size but in this case were located within the same postal zip code. Each postal zip code area generally encompasses about 5-10 square miles of the total 980 square miles that Lancaster County comprises. (Table 3 and Table 4)

The results of 2016 milk production comparison shows the only significant differences to be yearly herd production level and the somatic cell count scores. While conventional production had significantly better somatic cell count scores, both groups had a linear somatic cell count score below <100,000 (linear SCC 3), well below the generally accepted standard for good quality milk of somatic cell count of 400,000 (linear SCC 5).

With the above results it would be good to describe the treatments that the organic herds are using for common maladies such as mastitis and infertility. The data shown for certified organic herds includes only animals that are in the herd. No animals treated with antibiotics and/or hormones are in the certified organic data set presented above.

Mastitis

With prohibitions on antibiotics and hormones, it is imperative to rely on the animals' immune systems rather than antibiotics. Three important components will determine immune response to challenges. First is the animal's genetic resistance or susceptibility to specific pathogens. Secondly, when the diet is complementary to the animal's digestive system, the immune system will be able to respond efficiently. Thirdly, stimulation and modulation of the immune system is critical to regain health. In addition to genetics, diet and immune enhancement, the following factors generally ensure a healthy animal: (1) clean, dry bedding (2) fresh air and proper ventilation, (3) plenty of high quality water, and (4) direct contact with pasture/earth. The author has found that dairy cattle given pasture and high forage diets tend to have dramatically fewer health issues than those confined to concrete and fed diets heavy with grains to maximize production. The author's experience as a clinical practitioner bears out the same results in regards to dramatically less systemic illness in herds that utilize management-intensive grazing during the growing season, feed high forage diets throughout the year, and provide dry bedding and fresh air.

The use of biologics, botanicals and anti-oxidants are rational approaches to utilize when not using antibiotics and hormones. When considering human medicine and veterinary medicine, biologics and botanicals were relied upon prior to the advent of antibiotics and synthetic hormones. Vaccines can also be an important factor in helping the immune system respond rapidly to viral and bacterial challenges that may arise during an animal's life.

Biologics are compounds derived from living materials such as soil, plants, animals, fungi, bacteria and viruses. Products derived from these sources can affect both the non-specific and specific branches of the immune system. Products which affect the non-specific, or innate, immune system generally increase activity of interferon, which then stimulates natural killer cells and macrophages, which non-selectively kill pathogens. Passive antibodies, such as IgG antibodies which are created by a donor animal and then administered to a recipient animal, quickly target and efficiently eliminate pathogens in highly specific ways. Vaccines, the most ubiquitous biologics, stimulate the recipient to create antibodies in a preventive manner so that a rapid response is mounted by the animal when challenged by the pathogens for which it was vaccinated. In the author's experience, animals with coliform mastitis respond well if antibodies are administered within 12 hours of the onset of symptoms.

Conditions such as localized mastitis (not systemic coliform mastitis) and elevated somatic cell counts need to take into account milking hygiene and technique prior to considering treatment. This holds true for both conventional systems that use antibiotics as well as organic systems which prohibit antibiotic usage. While spontaneous self-cure can occur (depending on extent of challenge and immune capability of the animal) there are non-antibiotic treatments which appear to effectively resolve the problem quickly. Utilizing biologics is one method. While there are commercial vaccines available to effectively prevent and/or reduce severity of coliform mastitis symptoms, commercial vaccines for other types of mastitis aren't as common. For mastitis pathogens including staph and strep, an autogenous vaccine created directly from the milk of the specific herd is possible and in the author's experience has worked well in herds with a Staph aureus problem, if closely adhering to the vaccine program protocol, which starts at 6 months of age, and administering boosters at various times throughout the life of the animal. Other biologics include products derived from the colostrum of specifically stimulated, immunologically mature animals. This is a rational approach since colostrum is rich in antibodies. Biological products used against mastitis are generally injected subcutaneously and may help to stimulate IgA. This is smart since IgA is known as an antibody type that monitors mucosal membranes.

Botanical medicines are also available for mastitis to replace antibiotics. For instance, there are many commercially available topical ointments and sprays which can be applied to cows' udders against mastitis. Many popular ointments that are anti-inflammatory and soothing to a swollen udder contain Japanese peppermint mint (*Mentha japonica* and/or other mentha species). Farmers often use these as a first line of defense when milk quality is of concern. There is widespread testimonial and anecdotal evidence regarding such ointments. Since the teat orifice of a cow is very easy to infuse with medication, dairy farmers (both organic and conventional) will often infuse treatments other than antibiotics for mastitis. Using non-antibiotic treatments is becoming more common and popular due to highly sensitive tests that can pick up minute traces of antibiotics in milk and result in significant penalties to a farmer. North Carolina State University has done clinical trials with commercially available botanical infusions. A multi-ingredient botanical product called Phyto-Mast™ has been studied extensively. The active ingredient is thymol, balanced with angelica, gaultheria, and glycyrrhiza, in a base of organic olive oil. In an in vitro study done on its ingredients and their effect on pathogens, it was shown that thymol at a concentration of greater than 2% consistently resulted in no bacterial growth of Staph. aureus, Staph. chromogenes, and Strep. uberis.⁴ In a study with lactating

cows, it was shown that treatment with the botanical preparation Phyto-Mast™ resulted in a reduction of the time to clinical recovery.⁵ In a study on drying off cows, the efficacy was similar to that of conventional (antibiotic) therapy, and there were no apparent adverse effects.⁶ As part of a PhD thesis (but not published in a peer-reviewed journal), it was shown that Phyto-Mast™ used in combination with Orbeseal™ had nearly identical cure and prevention of intramammary infections as published information with Quartermaster™ used in combination with Orbeseal™ (78% and 80%, respectively). Regarding the all-important issue of residues for food safety concerns, Phyto-Mast™ was detectable and quantifiable in goat plasma beginning with the 15-minute post treatment sample, but was no longer detectable in the 4-hour post treatment sample. Thymol residues were only detected in the 12-hour post treatment milk sample. An inflammatory response was not evident following phytoceutical administration.⁷ There is a 3 year USDA-funded study currently being done at North Carolina State University to describe the pharmacokinetics of three botanical products in lactating dairy cows: intramammary administration of Phyto-Mast™, an orally administered garlic tincture and a topically applied oregano oil. Garlic and oregano oil are very commonly used for illness in the organic dairy sector. When considering the current literature, Phyto-Mast™ is possibly the most scientifically studied natural treatment known.

Homeopathic remedies are another mode of treatment utilized on many organic farms. The author's experience as a herdsman treating organic dairy cows with homeopathic remedies was so successful that he went on to learn veterinary medicine in order to understand how such dilute remedies work. It quickly became evident that the immune system was involved. Homeopathic prescribing generally dictates that whatever symptoms a material in crude form can elicit, its derivative in highly diluted and vigorously shaken form can cure when a patient is ill and showing those same symptoms (the axiom of like cures like). While there are hardly any clinical studies with homeopathic remedies in dairy cows, there are simply too many anecdotal successes to dismiss this mode of therapy entirely. In fact, many of the herds in the Lancaster, Pennsylvania region that are included in the data presented in this paper rely on homeopathic remedies to some degree for milk quality concerns and fertility issues.

Some commonly used homeopathic remedies for high somatic cell count and clinical mastitis include apis, belladonna, bryonia, calc carb, calc phos, hepar sulph, lachesis, phytolacca, pulsatilla, silica, and sulfur. These medicines are commercially available as tiny medicated pellets and administered two to three times a day upon any mucosal membrane, usually given orally but also given vaginally. Homeopathic remedies can also be administered as liquids or sprays. A key concept when using homeopathic remedies is that selection of remedy is highly specific to the symptoms shown and that one needs to change remedies if symptoms change. When a clinical cure is observed, the need for remedy administration ceases. For instance, if a cow is exhibiting signs of mastitis with a hot, swollen, red udder and also has a pounding heart, dry oral membranes, and dilated pupils, homeopathic belladonna would be indicated and given hourly until those symptoms subside and/or change. (The crude form of *Atropa belladonna* if ingested would result in those same symptoms). In the author's experience, farmers that enjoy using homeopathic remedies tend to be extremely observant to subtle changes in the condition of their cows. When using standard antibiotic therapy, administration of the medicine requires a set amount of days in order to ensure adequate time to eliminate the bacterial pathogen challenge and observations of subtle changes are not necessary. Like many other natural treatments, homeopathy relies completely on enhancing the animal's own immune system to eliminate the infection whereas the primary aim of antibiotic therapy is only to eliminate pathogens.

An interesting hybrid approach to the biological and homeopathic methods is to make an autogenous vaccination by extracting the animal's own blood, quickly mixing in a specific homeopathic remedy, and re-injecting the mixture subcutaneously to the same animal. This method is

Variable	Conventional	Organic	P value
Total Cows	40	39	.497
Rolling Herd Average	21,745	16,251	.000
Fat %	3.61	3.67	.163
Protein% (n=28)	2.91	3.03	.089
Actual SCC	322,735	331,617	.755
SCC <40 DIM (n=33)	2.57	3.11	.143
SCC 41-100 DIM (n=33)	2.21	2.49	.314
SCC > 305 DIM (n=32)	3.46	3.58	.687
SCC 1 st lactation (n=33)	2.35	2.46	.584
SCC 2 nd lactation	2.53	3.06	.015
SCC 3+ lactations	3.27	3.47	.218

Table 1: Comparison of production between conventional and certified organic herds (2006)²

Variable	Conventional	Organic	P value
Average age at 1st calving (n=33)	25.0	26.0	.041
Calving Interval (months)	14.2	14.0	.307
Days to 1st service (n=32)	90.6	88.4	.668
Days open	152.5	146.8	.318
Services per pregnancy	2.92	2.56	.109
Cull rate (n=33)	33.4	26.6	.004

Table 2: Comparison of reproduction between conventional and certified organic herds (2006)²

Variable	Conventional	Organic	P value
Total Cows (n=67)	49	48	.65
Rolling Herd Avg (lbs) (n=67)	19,708	16,417	<.01
Fat % (n=67)	3.7	3.9	.11
Protein % (n=64)	3.1	3.0	.71
Linear SCC (n=64)	2.6	2.8	<.01
Actual SCC (x 103) (n=64)	221,000	269,000	<.01
SCC < 40 DIM (n=61)	3.3	3.7	.11
SCC 41-100 DIM (n=64)	3.0	3.4	.11
SCC > 305 DIM (n=60)	3.5	3.6	.73
SCC Lactation 1 (n=64)	2.9	3.0	.56
SCC Lactation 2 (n=64)	3.1	3.5	.06
SCC Lactation 3 (n=64)	3.9	4.1	.18
SCC Lactation 4 (n=61)	3.3	3.7	.11

Table 3: Comparison of production between conventional and certified organic herds (2016)³

Variable	Conventional	Organic	P value
Conception Rate (n=59)	47.2	49.2	.55
Pregnancy Rate (n=59)	18.6	18.6	.55
Percent Observed Heats (n=59)	41.7	31.2	<.01
Days to 1 st service (n=67)	87	47	<.01
Days open (n=67)	143	101	<.01
Calving Interval (n=67)	13.5	13.4	.43
Cull rate (involuntary) (n=65)	26.9	24.8	.27
Avg. age (n=65)	47.5	49.7	.10

Table 4: Comparison of reproduction between conventional and certified organic herds (2016)³

called an autosanguis, a therapeutic procedure utilized by practitioners of homotoxicology, a derivative of homeopathy which applies a broad range of homeopathic remedies in various potencies to affect physiologic processes. A commercial example of a homotoxicologic product which is known around the world is Traumeel™ (Heel GmbH). Though not traceable through titer changes, correctly selected homeopathic remedies appear to resolve various conditions, if only ascertained through individual clinical cure. No doubt some portion of the data presented for certified organic farms is associated with homeopathic remedies being utilized.

Reproduction

The results of the most recent study (2016) shows the only significant differences are in observed heats, days to 1st service and days open. While the organic herds had significantly better levels in these three categories, the results may be due to more use of bulls rather than artificial insemination. The data presented in the tables show that while there are some significant differences at the herd level between reproduction parameters of conventional and certified organic herds, there are also no differences in key reproduction indicators. One key indicator where there are no significant differences is pregnancy rate. In the United States, there is widespread use of synchronized artificial insemination using protocols involving multiple injections of prostaglandin $F_{2\alpha}$ and gonadorelin (PG) releasing hormone (GnRH). These two hormone compounds are prohibited from use in certified organic livestock in the United States. Aside from the use of more bulls to reduce days to 1st service and days open while reducing observed heats, it is intriguing to look at modes of treatment for reproduction and fertility issues in certified organic herds. Certainly the first two weeks post-partum are critical to make sure the uterine environment will be ready for timely conception. In cases of metritis, placement of antiseptics is needed starting about day 3 post-partum until the cervix closes. Manual removal of placenta at approximately day 5 or 6 post-partum is critical. Antiseptic compounds such as iodine (1 gram dry or equivalent in liquid volume) are beneficial if given daily before the cervix closes. Other products include essential oils (mixed within powder and placed in capsules), colostrum-whey infusions mixed with botanicals such as calendula tincture, aloe lavages, and dilute chlorhexidine. The key is to make sure that there is daily treatment prior to the cervix closing. Oral administration of homeopathic pyrogenium is often utilized. In fact pyrogenium is derived from rotten meat, which is what a retained placenta is; thus, pyrogenium is a perfect homeopathic match for the condition of retained placenta. In treating pyometra, infusing the uterus with a mixture of 3 parts of dextrose or 3 parts of hypertonic saline with 1 part 7% iodine tincture has helped to clear many low grade pyometra cases if treated within the first 3-4 months post-partum. The volume infused is based on the size of the pyometra. For instance a pyometra that is the size of a 60 day pregnancy would be treated one time with 60 cc of the mixture. Whereas a pyometra that is the size of a 90 day pregnancy is treated with 90cc of the mixture two times one week apart. A 120 day "pregnancy" is best treated with 120cc twice weekly for 2-3 weeks. Larger pyometra is often not responsive to treatment.

Cows that are physiologically normal but aren't showing behavioral estrus are prompted into behavioral estrus with a powdered botanical phyto-estrogenic combination of *Angelica sinensis*, *Turnera diffusa*, *Oenothera biennis*, *Dioscorea villosa*, *Mitchella repens*, *Viburnum opulus*, *Linum usitatissimum*. The combination is given orally beginning at mid-cycle and given daily for six days in a row or until heat, whichever is first. Though no data exists for this use, the author's clinical impression is that this treatment brings correctly selected cows into behavioral estrus >80% of the time. Cows do not abort if given this treatment by mistake, as it is phyto-estrogenic and not luteolytic. Consequently this herbal combination is not effective for treatment of pyometra as is PG.

Conclusion

The need to look more closely at non-antibiotic and non-hormonal therapy as a primary mode of treatment has been stimulated by the "no antibiotics" rule in the United States certified organic livestock sector. Epidemiologic cross-sectional studies can give clues as to what may be working even though specific cause and effect studies are lacking. Further clinical studies are needed to provide the data necessary to help reduce antibiotic use in livestock agriculture.

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Diagnostic imaging in bovine medicine

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Introduction

Diagnostic imaging procedures such as ultrasonography, radiography, computed tomography (CT), magnetic resonance imaging (MRI), endoscopy and infrared thermography have become widely used in bovine medicine in the past decades particularly in veterinary teaching hospitals. However, CT and MRI are still limited to well-equipped institutions because of their high costs and the necessity to use general anesthesia (Nuss et al., 2011c). Furthermore, a radiographic unit is not standard equipment for bovine practitioners (Kofler et al., 2014). The same conditions apply for the use of endoscopes, even though teat endoscopes are used routinely in specialized practices (Geishauser et al., 2005).

Non-invasive ultrasonography, on the contrary, evolved into a valuable diagnostic imaging technique in almost all fields of bovine medicine (Table 1, 2). This is most probably due to the development of technically improved, cost effective portable units allowing its use at any given location (King, 2006).

The first goal of this presentation is to review the various technical opportunities of diagnostic imaging, the second goal is to point out the wide range of applications of ultrasonography in bovine practice using commonly available units equipped with 5 – 8 MHz rectal probes. We would like to encourage veterinarians to use diagnostic ultrasound for various other indications beyond the examination of the reproductive tract.

Preceding clinical examination

A careful physical, gynecological/andrological, orthopedic, and neurological examination of the patient is always the first step in making a clinical diagnosis before imaging techniques are used to achieve a definite diagnosis (Baumgartner, 2014). This thorough clinical examination reveals the “organ/region of interest” for the subsequently applied diagnostic imaging technique (Kofler & Hittmair, 2006).

Ultrasonography

Ultrasonography has been globally established as a routinely applied diagnostic imaging method in bovines. The female reproductive tract was the first organ system studied by ultrasound and this application became widespread in ambulatory practice (Pierson & Ginther, 1988; DesCôteaux et al., 2009; Mee et al., 2009; Fricke et al., 2016). Nowadays, ultrasound is an integral part of diagnostics in gynecology, andrology, internal medicine, cardiology, orthopedics and neurology in many veterinary teaching hospitals (King, 2006). The person undertaking the clinical evaluation usually also performs the ultrasonographic investigation. The advantage of this union is that the operator is well informed about the anatomical sites in question and is therefore able to relate these to the previously determined clinical findings (Scott, 2008, 2009).

Making a definite clinical diagnosis with abdominal, thoracic, cardiac, umbilical, urogenital, gynecological and musculoskeletal disorders is often challenging or impossible. Ultrasound is superior to radiography for the diagnosis of conditions affecting soft tissues (Table 2). It is ideal

for the evaluation of musculoskeletal disorders and other swellings (Kofler, 1996a,b; Nuss & Maierl, 2000; Nuss, 2003; Nuss et al., 2007, 2011a; Altenbrunner-Martinek et al., 2007; Kofler & Altenbrunner-Martinek, 2008; Gansohr et al., 2009). Even bone surfaces that are difficult to assess radiographically such as the scapula, ribs and pelvis, as well as the bones of the limbs can be imaged ultrasonographically for the presence of fractures, osteitis and osteomyelitis (Kofler, 1997a,b; Grubelnik et al., 2002; Kofler et al., 2014).

Ultrasonographic examinations should adhere to a standardized protocol (Kofler & Hittmair, 2006; Braun, 2009a-c; Ollivett & Buczinski, 2016). With whose assistance, the sonographer examines the organ/structure of interest in a certain sequence in order not to overlook lesions or conditions. Additionally, each organ should be imaged in transverse and longitudinal views (O'Brien & Forrest, 2005; Buczinski, 2009b).

The ultrasound probe is used by the sonographer much like the own fingers for sonopalpation (Kofler & Hittmair, 2006) examining the contents of body and synovial cavities, cysts, vessels or other fluid-filled lesions for the presence of liquid, semi-solid, or solid contents and/or inflammatory effusion. Flow phenomena are tested by applying forced pressure to a body cavity showing effusion with either the transducer or the contralateral hand and verifying flows on the monitor image (Kofler & Hittmair, 2006).

In addition, sonopalpation is employed for the diagnosis of thrombus formation and differentiation of arteries and veins. Normal veins can be compressed with the transducer and pulsed flow is identified in arteries (Kofler, 1995a; Braun & Forster, 2012; Braun et al., 2013). The main vascular diseases in cattle include inflammation of the vessel walls, thrombosis, and aneurysm (Kofler et al., 1996, 2002; Bollwein et al., 2000; Braun & Föhn, 2005; Buczinski, 2009a). Colour Doppler sonography had been established as a useful technique for the investigation of the genital blood flow during all reproductive phases (Herzog & Bollwein, 2007; Krueger et al., 2009; Waldvogel & Bleul, 2014; Heppelmann et al., 2013; Bollwein et al., 2016).

Centesis of body and synovial cavities, puncture of the subarachnoid space, biopsy of organs for cytologic and/or histologic analysis and transvaginal puncture of ovarian follicles is frequently applied (Heppelmann et al., 2013, 2015; Braun, 2016a; Braun & Attiger, 2016). Following ultrasonographic assessment of the designated structure, it offers guidance for the needle placement, assisting with the measurement of the distance from the skin surface to the structure. This is especially important if a freehand or an ultrasound-guided needle placement is performed (King, 2006; Braun et al., 2009a,b; Tharwat et al., 2012; Braun, 2016a). A new clinical application is the ultrasound-guided perineural anesthesia of proximal limb nerves (femoral, sciatic, paravertebral nerves, brachial plexus) as an alternative to general anesthesia (Iwamoto et al., 2012; Shokry & Berbish, 2012; De Vlamynck et al., 2013; Kramer et al., 2014; Re et al., 2014, 2016). Furthermore, 6–10 MHz linear transducers had been applied as a useful ancillary tool for assessing spinal cord diseases (Testoni et al., 2010a,b; 2012, 2014; Braun & Attiger, 2016).

The costs for purchase and up-keep of a sonographic unit is just too high to limit its application to the bovine reproductive tract. In calves in particular, nearly all organ systems can be examined with 5 – 8 MHz rectal linear probes (Table 1, 2), and even in cows these rectal probes can be used for imaging of pathologies of many joints, the teat, pleura and the lung, the reticulum (mainly in young cattle), umbilical region, large vessels and for determination of the back fat thickness (Cartee et al., 1986; Lischer & Steiner, 1994; Kofler & Edinger, 1995; Braun et al., 1997; Rabeling et al., 1998; Flöck, 2003, 2004; Klein et al., 2005; O'Brien & Forrest, 2005; Flöck & Winter, 2006; Joshi & Herdt, 2009; Rings, 2009; Steiner & Lejeune, 2009; Hussein et al., 2013; Buczinski et al., 2013, 2014; Braun, 2016b; Ollivett & Buczinski, 2016).

The issue of the Veterinary Clinics Food Animal from 2009 entitled “Bovine Ultrasound” (Fig. 1) covers the examination technique and the

ultrasonographic findings of the gastrointestinal tract, liver, umbilical region, the cardiovascular, respiratory and musculoskeletal system, urinary tract, udder and teat, the female and male reproductive tract (Braun, 2009a,b; Buczinski, 2009a,b; Babkine & Blond, 2009; Flöck, 2009; Franz et al., 2009; DesCôteaux et al., 2009; Gnemmi & Lefebvre, 2009; Steiner & Lejeune, 2009; Kofler, 2009). This issue and the recently published issue entitled "Update on Ruminant Ultrasound" (Fig. 2) are highly recommended as helpful guides for clinicians interested to expand their diagnostic horizons (Ollivett & Buczinski, 2016; Mitchell & Schwarzwald, 2016; Braun, 2016a,b; Bernier Gosselin et al., 2016; Re et al., 2016; Bollwein et al., 2016; Fricke et al., 2016).

Many journal articles also give an overview of the ultrasonographic examination and the pathologies of the musculoskeletal system (Kofler, 2009, 2011; Kofler et al., 2014), the sole horn and the digital fat pad thickness (Kofler et al., 1999; Bicalho et al., 2009), the reproductive tract and mammary gland (DesCôteaux & Buczinski, 2009), pathologies of the retropharyngeal region (Braun et al., 1994; Gansohr et al., 2009; Rings, 2009), heart and pericardium (Flöck & Baumgartner, 2001; Braun et al., 2001; 2007a; Braun, 2009c; Buczinski et al., 2010), lungs (Braun et al., 1997; Rabeling et al., 1998; Jung & Bostedt, 2004; Scott, 2009, 2012, 2013b; Buczinski et al., 2013, 2014), gastrointestinal tract (Braun, 1997, 2003; Braun et al., 2007c; Braun & Feller, 2008; Braun et al., 2008a, 2010, 2011; Kumar & Saini, 2011; Braun & Gautschi, 2013), liver and spleen (Nagaraja & Lechtenberg, 2007; Braun & Krüger, 2013; Scott, 2013a; Abdelaal et al., 2014), kidneys (Braun et al., 2008b; Abdelaal & Flöck, 2015), pancreas (Tharwat et al., 2013) and the abdominal fat necrosis (Tharwat & Buczinski, 2012).

Radiography

During the last half century radiography has been established as a standard diagnostic procedure in bovine medicine. Nevertheless, up to now it has not become a routine procedure in bovine practice, particularly due to the high investments and the rather low frequency of application in practice (Kofler et al., 2014). Until now, only few textbooks and articles have been published about radiography such as these two references: the textbook of "Bovine Radiology" by Bargai et al. (1989) and the recently published DVD "Bovine Radiology – Digital Diagnostic Atlas" from the Vetsuisse-Faculty in Berne (Fig. 3; Steiner et al., 2010). A detailed overview about radiography and ultrasonography in bovine orthopedics was given by Kofler et al. (2014).

Radiography remains the method of choice to image bony structures and the location and type of abnormal masses (Table 1). However, any radiological examination must yield a benefit sufficient to justify the risks of the radiation exposure (Steiner et al., 2010; Kofler et al., 2014).

One of the most frequent indications for bovine radiography (Table 1) is the definite fracture diagnosis, because the type of surgical treatment is mainly based on the fracture configuration (Steiner et al., 1993; Bellon & Mulon, 2011; Nuss et al., 2011b; Wenzinger et al., 2012; Vogel & Anderson, 2014). Other important indications are the diagnosis of luxation, osteitis, osteomyelitis and advanced stages of septic arthritis (Van Huffel et al., 1989; Verschooten et al., 2000; Starke et al., 2006, 2007; Braun et al., 2009a; Desrochers & Francoz, 2014; Kofler & Peterbauer, 2014) and the diagnosis of vertebral column disorders (Zani et al., 2008; Muggli et al., 2011).

The use of radiography for the diagnosis of lung disorders and for traumatic reticuloperitonitis in young and adult cattle which had been used frequently in the past is used rather rarely today (Verschooten et al., 1974; Bellavance et al., 2010; Siegrist & Geisbühler, 2011), while nowadays it had been widely supplemented or replaced by ultrasonography (Babkine & Blond, 2009; Braun, 2009c; Buczinski et al., 2010).

CT and MRI

Computer tomography (CT) is a specific, computerized type of X-ray examination. The most important advantage of CT versus conventional radiography is that CT provides sectional images of body regions without being obscured by overlaying structures (Nuss et al., 2011c). One main benefit of Magnetic resonance imaging (MRI) versus CT is the much more detailed contrast imaging of soft tissues (Zani et al., 2008; Wuersch et al., 2009). Nevertheless the high costs, these imaging techniques can be an option in highly valuable cattle (Steiner, 2012) for achieving a comprehensive diagnosis, which cannot be made by radiography and ultrasonography, and for preoperative planning of a complex surgical intervention (Nuss et al., 2011c; Sparks et al., 2014). However, technical restrictions such as the size of gantry aperture of the CT and the MRI units limit the applications in adult cattle to the examination of the head, cranial parts of the neck and the limbs (Ehlert et al., 2011; Nuss et al., 2011c). In contrary, in calves depending on their size and age the entire body can be scanned (Braun et al., 2014a-c; Ohlerth et al., 2014).

Indications for the use of CT and MRI have been reported and include mainly anatomical studies of bovine limbs (Tiefenthaler, 1997; Schwarze, 1998; Kofler et al., 1999; Ehlert et al., 2011; Raji et al., 2008; 2009; El-Shafey & Sayed-Ahmed, 2012; Tsuka et al., 2012, 2014) and the development of stomach in calves (Flor et al., 2012). Only rarely clinical applications of these methods were reported such as the evaluation of head disorders such as tympanic bulla in calves with otitis-media (Finnen et al., 2011; Lee et al., 2011; Bernier Gosselin et al., 2012), dental, nasal and sinusoidal diseases (Nuss et al., 2011c), atrophic rhinitis (Frame et al., 2000), actinomycosis (Mohamed et al., 2011), brain and spinal epidural abscesses, multiple pulmonary and pharyngeal abscesses and hydrocephalus in calves (Zani et al., 2008; Lee et al., 2009, 2011), nasal osteoma (Wuersch et al., 2009), orthopedic diseases and congenital malformations (Willems & Dik, 1995; Lee et al., 2009). Recently a research group from Switzerland established CT reference values of the thoracic and abdominal organs in calves from birth to 105 days of age (Ohlerth et al., 2014; Braun et al., 2014a-c). Table 1 gives an overview of reported indications for CT and MRI in bovines.

Endoscopy

Endoscopic techniques are minimally invasive, and they can be applied in many cases in the standing and restrained animal requiring sedation and local anesthesia depending on the region (Franz, 2012). The direct visualization of inner organ surfaces allows the immediate identification of pathologies, and thus to state a definite diagnosis and to give a substantiated prognosis in many patients (Anderson et al., 1993, 1994; Franz et al., 2004; Franz, 2011; Mann et al., 2013).

Table 1 gives an overview of the most important indications for endoscopy in bovines. Parts of the upper respiratory tract including nasopharynx, larynx, trachea and the two main bronchi (Kahl & Hofmann, 1985; Pringle et al., 1988; Anderson et al., 1994; Dadak et al. 2009; Gansohr et al., 2009; Sydlar et al., 2013; Prohl et al., 2014), the oesophagus (Franz & Baumgartner, 2002; Franz et al., 2006b; Mann et al., 2013), parts of the gastrointestinal tract (Anderson et al., 1993; Steiner & Zulauf, 1999; Klein et al., 2002), the urethra and urinary bladder (Franz et al., 2004; Braun et al., 2007b, 2009b; Chiesa et al., 2009), the abdomen (Wilson & Ferguson, 1984; Rommel et al., 1990; Anderson et al., 1993; Franz et al., 2000), the teat (Medl et al., 1994; Seeh & Hospes, 1998; Sheakespeare, 1998) and the epidural canal (epiduroscopy) (Franz et al., 2008) are all accessible by means of endoscopy. In contrast, rumenoscopia, hysteroscopia and epiduroscopia are to date only of experimental interest (Franz et al., 2006a; Madoz et al., 2010; Franz, 2012).

Theloscopy represents the "gold standard" for direct visualization of the teat canal, the rosette of Fürstenberg, and the teat cistern (Hospes & Seeh, 1999), and is combined with the subsequent surgical intervention in cases of disturbance of milk flow, since mucosal tissue can be removed under visual control in specialized bovine practices too (Medl

et al., 1994; Hirsbrunner et al., 2001; Geishauser et al., 2005).

A further indication for endoscopy is the visually-aided biopsy collection from the liver, abdominal lymph nodes, spleen, kidneys, intestine (Naoi et al., 1985; Klein et al., 2002; Chiesa et al., 2009) and the extraction of fluid samples as described for the bronchoalveolar lavage (Prohl et al., 2014).

Therapeutic applications for endoscopy in cattle are arthroscopy and tenovaginoscopy (Gaughan, 1996; Bertagnoli et al., 2012; Lardé & Nichols, 2014). Another important field for therapeutic endoscopy is the treatment of left displaced abomasum by repositioning and fixation to the abdominal wall under endoscopic control (Newman et al., 2008; Wittek et al., 2009). A comprehensive and detailed overview regarding the examination technique, normal and pathological findings in bovine endoscopy is available online by the Digital Atlas of endoscopy in cattle (Fig. 4) (Schlup et al., 2010).

Infrared thermography (IRT)

IRT has been introduced recently in bovine medicine to identify and determine thermal abnormalities by characterizing an increase or decrease in the surface temperature of their skin at an individual and herd level (Wood et al., 2015). Changes in blood flow can be used to detect inflammation or injuries associated with conditions such as subclinical and clinical mastitis (Hovinen et al., 2008; Polat et al., 2010; Metzner et al., 2014, 2015), to detect lameness early (Alsaad et al., 2015) and to identify foot lesions (Alsaad & Büscher, 2012; Stokes et al., 2012; Alsaad et al., 2014; Wilhelm et al., 2015). Further new applications intended to develop algorithms for assessing the ability of an IRT device for estrus detection and as a predictor of time of ovulation in cows (Talukder et al., 2014), to assess seasonal effects of the environment on semen quality in bulls (Menegassi et al., 2015), and to use IRT for automated detection of bovine respiratory disease onset in receiver calves (Schaefer et al., 2012) (Table 1). However, this technique is highly subjected to environmental thermal artefacts (wind, humidity, exposure to light), so that a controlled environment is a strict precondition before image scanning (Wood et al., 2015). Until now none of these described indications has been established as a routine examination procedure in cattle.

Conclusions

In combination with the assessed clinical findings diagnostic imaging techniques are of great importance for making an exact diagnosis. In some countries, bovine practitioners can refer bovine patients to veterinary teaching hospitals. However, many practitioners use rectal ultrasonographic units for the examination of the reproductive tract, and these 5–8 MHz linear probes are in addition well suited for differentiation of soft tissue swelling (arthritis, tenosynovitis, bursitis) in the limbs (Kofler, 2009; Kofler & Martinek, 2004; Heppelmann et al., 2009). Furthermore, the same rectal probes are well suited for examination of a wide range of other organs, especially in calves but also in adult cattle, where the ultrasound wave penetration of these frequencies is enough to investigate some lung and pleural, abdominal, umbilical disorders or swellings elsewhere in the body (Flöck, 2007; Babkine & Blond, 2009; Franz et al., 2009; Steiner & Lejeune, 2009; Ollivett & Buczinski, 2016; Braun, 2016a,b) and for examination of the spinal cord (Testoni et al., 2012, 2014; Braun & Attiger, 2016).

Regardless of the applied diagnostic imaging techniques, an accurate anatomical differentiation of the (soft) tissue structures involved, characterization of the lesions, an early diagnosis, and a thorough preoperative inspection of incriminated region are of enormous benefit for determining an accurate prognosis as well as planning surgery and treatment and/or decision making (Braun, 2005; Braun et al., 2008b, 2011; Kofler et al., 2014; Ollivett & Buczinski, 2016; Braun, 2016a,b) in bovine patients.

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For tables and references, please go to the following webpage: <http://imgpublic.mci-group.com/ie/PCO/JohanKofler.pdf>

RESET the mindset on antibiotic usage in dairy cows

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Since the second world war, antibiotics came widely available to cure bacterial diseases, which had an enormous impact on lifetime expectation of diseased people. In the animal industry antibiotics were also used, initially to cure diseases, later also to prevent diseases and as growth promoters. The availability of antibiotics was of great importance for the improvement of animal health in the dairy industry, specifically related to udder health and to a less extent in claw health and uterine diseases. Due to the simple fact that antibiotics have an effect on the production process of products such as cheese and yoghurt, antibiotic use in lactating cows has always been limited.

In 2008 antibiotic usage in the livestock industry became a political issue in the Netherlands. Whereas antibiotic usage in humans was relatively low compared to other European countries, antibiotic usage in the Dutch livestock industry was relatively high. After a number of incidents with methicilline resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta-lactamases (ESBL) in animals, reduction goals for antibiotic use in animals were set by our government. The goal set was a decrease with 20% in 2011, 50% in 2013, followed by 70% in 2015, as compared to 2009. At the same time it was indicated that the livestock industry itself had the responsibility to realize this reduction. In that same year a national taskforce was established in which the major livestock sectors participated.

At that time it was clear that, although the dairy sector was not the sector in which most antibiotics were used, there were some issues there too which had to be solved. The challenge in the dairy industry was to realize a reduction in antibiotic usage although usage was relatively low already. Over the years blanket dry cow therapy and extended treatment of (sub) clinical mastitis had been fiercely promoted, zero-withdrawal products were widely available and used. All of these were considered to have a positive effect on health and welfare, which we did not want to lose. What the specific issues were, what decisions were made and how we managed to reset the mindset of farmers and veterinarians, is described in this paper.

Antibiotic resistance in cattle

With respect to antibiotic resistance in dairy cattle, there is a huge difference between udder pathogens and other pathogens. It seems logic to focus on udder pathogens, because antibiotics are mainly used intramammary. Most bacteria, however, live in the intestinal tract, and that is where potentially most antibiotic resistance will develop.

In udder pathogens, antibiotic resistance is most prominent in Gram-positive bacteria, specifically staphylococci. Once in a while an ESBL will be found in milk, but that generally is considered as a contamination from the environment, rather than being an intramammary pathogen.

Resistance to methicilline in *Staph. aureus* is an issue related to penicillin resistance. Based on oxacillin testing and confirmed by the presence of the *mecA*-gene, MRSA has been monitored in the Netherlands since 2007. In 2008 a prevalence of MRSA of 2% was found in a prospective prevalence study in 200 dairy herds (Olde Riekerink et al., 2010). A total of 0.2% of the tested cows was positive on MRSA. All strains belonged to Spa-type t011 (ST398), indicating they were livestock-associated MRSA (Tavakol et al., 2012). These findings indicate that the prevalence of

MRSA in dairy milk is low as compared to for instance pigs (de Neeling et al., 2007).

With respect to Gram-negative bacteria, ESBL producing *E. coli* were found in a low number of milk samples (Hordijk et al., 2013). In faecal samples of young calves, however, ESBL prevalence is higher and comparable to other species. We currently are evaluating the dynamics of ESBL in calves and its relation with antibiotic usage in calves and in dry cow treatment.

Issues on antibiotic usage in the dairy industry

Most of the antibiotics used in dairy cows are used intramammary. In the Netherlands this used to be approximately 70% of total antibiotic usage of which roughly 1/3 in mastitis treatment and 2/3 in dry cow treatment (Kuiper et al., 2016). Because of withdrawal-time of antibiotics used to treat mastitis in lactating cows, this usage likely is as limited as possible. Of course udder health can be improved in many situations, but given the effort done in this field over the last years in our country, no major gains are expected here. For dry cow therapy that is different. From 2013 onward, blanket dry cow therapy (BDCT) was no longer allowed in our country, where it had been fiercely promoted in earlier years. It was expected that selective dry cow therapy (SDCT) would lead to an increase of clinical and subclinical mastitis (Scherpenzeel et al., 2014). In 2014 a guideline was launched by the Royal Dutch Veterinary Association, which stated that dry cow antibiotics were only allowed after intramammary infections (IMI) were diagnosed at drying off. As indication of IMI, somatic cell count (SCC) could be used. It was found that the cut-off levels of SCC did have a limited effect on the incidence of clinical and subclinical mastitis at the herd-level (Scherpenzeel et al., 2016). In spite of a reduction in the usage of dry cow antibiotics of approximately 28% in 2014, according to the Dutch Veterinary Medicine Authority, udder health did not decline enormously (Santman-Berends et al., 2016). In a thorough questionnaire it was found that in 2013, before they had much experience with it, most farmers were convinced that SDCT, and the selective use of antibiotics in general were a sound approach.

Apart from the quantitative approach, the types of antibiotics used also became important. Antibiotics used in the animal industry were subdivided in three categories, with increasing likeliness to give rise to development of antibiotic resistance. First preference antibiotics are considered to be effective for the specific indication and don't induce resistance by ESBL/AmpC production. Second preference antibiotics are only used when unavoidable. There has to be a specific documented reason like patient-history, sensitivity pattern or clinical urgency to use these antibiotics. Third preference antibiotics are those antibiotics that are of importance for treating multiresistant bacteria in human patients, such as 3rd and 4th generation cephalosporins, some fluoroquinolones and modern long acting macrolides. The use of third preference antibiotics is only allowed for individual animals, when bacteriological culture and sensitivity patterns showed there is no alternative.

The relation between antibiotic usage and antibiotic resistance in veterinary medicine has been shown in general (Chantziaras et al., 2014) as well as for specific antibiotics. In the United Kingdom it was found that herds that used 3rd or 4th generation cephalosporins were almost four times more likely to have ESBL producing *E. coli*, while no association was found with other antimicrobials (Snow et al., 2012).

Data on the usage of the different types of antibiotics are monitored on a national base by the Dutch Veterinary Medicine Authority (SDa), which is an independent institute that calculates antibiotic usage and sets benchmark indicators for the different animal species. The SDa consists of a panel of experts with a background in microbiology and epidemiology, both human and veterinarian. Since the launch of the differentiation system in 2012, the amount of third preference antibiotics used in the dairy industry decreased enormously, as is indicated for 3rd and 4th generation cephalosporins in Figure 1.

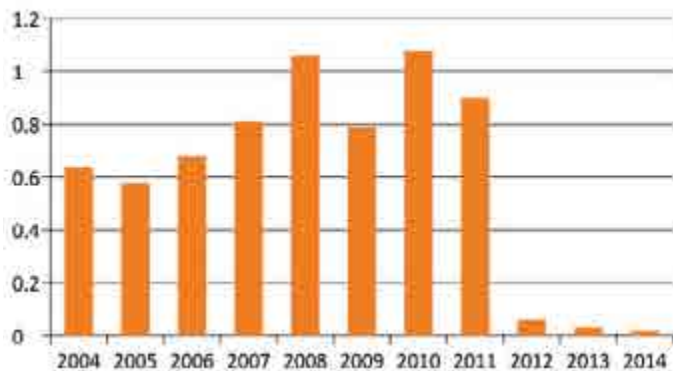


Figure 1. Use of third and fourth generation cephalosporins in the Netherlands in 2004-2014, expressed in animal defined daily dosages per year.

A final important aspect of antibiotic use in the dairy industry is residue handling. Consumption milk and milk that is processed are monitored very well, leading to a very low percentage of positive findings. In the past, however, very little attention was given to residues in waste-milk. This milk, that contained antibiotic residues in approximately 70% of samples (Heinrich et al., 2012) was often fed to young calves, in spite of the potential induction of antibiotic resistance (Brunton et al., 2012).

Changing the mindset

Traditionally veterinarians assume that dairy farming is an activity executed primarily based on rational, technical, and economic considerations (Burton, 2004). Although rational choices are crucial in farm management, we learned in mastitis studies that management is hardly ever fully rational (Jansen, 2010). One of the most used theories to understand people’s behaviour is the Theory of Planned Behaviour, described by Ajzen (1991) and by Fishbein and Yzer (2003). In short, this model says that if a farmer is actually willing to solve an issue, if he is positively influenced by important peers and if he has the feeling he can actually control and perform his actions, he will change his behaviour.

Based on the Theory of Planned Behaviour, the Health Belief Model (Janz and Becker, 1984), the Elaboration Likelihood Model of Persuasion (Petty and Wegener, 1999) and earlier work of van Woerkum et al. (1999) and Leeuwis (2004) we developed a model that is easy to use in practice, the RESET model (Figure 2).

This model fits very well as an explanation of the Dutch success in reduction of veterinary antimicrobial use, as described by Speksnijder et al. (2015). The different approaches used were found to be ingredients of the RESET model. It is important to use different approaches in a coordinated way to successfully influence the mindset. Because different people respond different to different stimuli, a ‘one size fits all’ approach assuming everybody will respond to it, will not work (Wessels et al., 2014). The behaviour of a group can therefore most effectively be changed using of a mix of different stimuli, which was done in the approach of decreasing antibiotic usage in the dairy industry in the Netherlands.

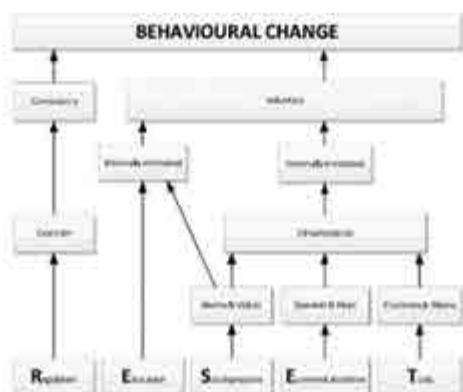


Figure 2. The RESET model (adapted from Van Woerkum et al. 1999, and Leeuwis, 2004).

The RESET model summarizes different models from literature in five important cues to action: regulation, education, social pressure, economic incentives and tools. Each may motivate a different group of people. With such a differentiated approach you hope to reach as much people as possible, trying to get over a tipping point.

The R of regulations, or rules force you to perform a desired behaviour. If rules are clear and if one is able to monitor whether rules are followed, they can be very effective. In the antibiotic discussion for instance, at some point it became clear that third preference antibiotics were no longer allowed. This was accepted and supervised by the SDa. The result is presented in Figure 1. Regulations, however, also are helpful for structure seekers, people who like to work according to a schedule that helps them to organize their work. The farm health and farm treatment plans as introduced in the Netherlands (Speksnijder et al., 2015) are examples of that.

A factor that traditionally is overestimated by veterinarians, but likely is underestimated in the antibiotics discussion, is education. Veterinarians generally are focused on teaching, delivering technical information, the rational approach (Burton, 2004; Jansen, 2010). The approach is that once a farmer will understand ‘how and why’, behaviour will change. This certainly is true for some farmers, but if it is the only approach, it will definitely not be sufficient. Interesting enough, in the antibiotics resistance discussion, the educative approach has hardly been used in the past. Many farmers did not know about the potential effects of the use of certain types of antibiotics on antibiotic resistance. All they talked about was efficacy, withdrawal time and prize. Veterinarians nor other advisors talked about the effect of suboptimal use of antibiotics on antibiotic resistance. Informing farmers on this issue certainly helped in reaching the numbers showed in Figure 1.

The probably most important factor in changing behaviour is social pressure. In most western countries society has changed in a few generations from a mainly agricultural society to one in which farmers are a minority. That has had its consequences on the way the general public thinks of farming and also on the way farmers think of farming. Those changes occur very slowly and differ from region to region, but they do occur. Once farmers stand up at meetings to tell about their experiences in hospital where they were isolated because they came from a farm, and explain they understand that, things are changing. Social pressure is also visible if farmers proudly tell in public meetings they have low antibiotic use in their herd, and are among the best in the benchmark. Exactly that is what happened in the Netherlands.

Although money certainly is not the only factor influencing behaviour, economics definitely is important. This is logic, because money is a major stimulus in the free-market economy. After the initial fear for the effect of taking away preventive antibiotics during the dry period, savings on expenditures on antibiotic use also influenced decision making by farmers.

Finally tools, technical facilities, also help in implementing behavioural change. The farm health and farm treatment plans are examples of this, as is the guideline on SDCT and the benchmark on antibiotic use at the herd level.

Conclusion

Antibiotic use and antibiotic resistance are important issues in the dairy industry, as they are in all parts of the livestock industry and in humans. For that reason, and because antibiotic use became a political issue, attention needed to be given to the subject. Since 2008 we earned experience on how to decrease antibiotic use in the Netherlands without leading to dramatic changes in udder health and in dairy health in general. We are convinced that cooperation of different stakeholders, being farmer organizations, dairy companies, veterinarians and others lead to a diversified approach that can be summarized in the RESET model. Because communication was taken seriously, taking into account that there is more than only rules, and combining multiple

strategies, the behavioural change regarding antibiotic use seems to be based on an actual change of mindset and therefore likely is successful on the long term.

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Metabolic disturbances in neonatal calves with diarrhoea – clinical signs and treatment

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Introduction

Neonatal calf diarrhoea remains one of the major health challenges in all bovine production systems. The infectious agents most commonly in diarrhoeic calves are *Cryptosporidium parvum* and rotavirus, followed by coronavirus and enterotoxigenic *Escherichia coli* (ETEC). ETEC usually only cause secretory diarrhoea in the first four days of life. The other common infectious agents involved in neonatal calf diarrhoea cause damage to the intestinal mucosa resulting in mixed malabsorptive and secretory diarrhoea. Irrespective of the causative agents the underlying pathophysiological mechanisms cause not only dehydration, but also several severe metabolic disorders. The purpose of this paper is to summarise the current knowledge on the clinical signs caused by the most important metabolic disturbances and their treatment.

Metabolic acidosis

Metabolic acidosis is a frequent complication of neonatal diarrhoea in calves and was traditionally attributed to intestinal losses of bicarbonate and an increase of blood L-lactate concentrations as a result of dehydration and concomitant tissue hypoperfusion.^{1,2} A well-known feature of metabolic acidosis in those animals is an increase of the anion gap, which indicates the presence of unmeasured anions when using the traditional Henderson-Hasselbalch approach. This increase could formerly not be explained by elevated L-lactate concentrations (Figure 1) and therefore resulted in an incomplete understanding of the pathophysiology of metabolic acidosis in neonatal diarrhoeic calves for a long time.³⁻⁵ However, this has been changed by the discovery that elevated blood D-lactate concentrations play an important role in the pathogenesis of metabolic acidosis in such animals⁶⁻⁸ (Figure 2).

The long-held view that faecal bicarbonate losses plays an important role in the development of acidaemia and metabolic acidosis in diarrhoeic calves has been questioned during the last decade.⁹ Application of the simplified strong ion approach to dehydrated diarrhoeic calves identified that acidaemia and strong ion acidosis was primarily due to a low strong ion difference as a result of hyponatraemia and an increase in unmeasured strong anions such as D-lactate. A non-volatile buffer ion acidosis, due to an increase in serum protein concentration was also identified to contribute to the acidaemia in dehydrated diarrhoeic calves.⁹

The simplified strong ion approach is based on Stewart's strong ion model^{10,11} and is conceptually differing from the traditional Henderson-Hasselbalch approach as it explains how 3 independent variables (partial pressure of carbon dioxide, the strong ion difference, and the concentration of nonvolatile weak acids such as albumin, globulin and phosphate) directly determine blood pH and bicarbonate concentration. A useful clinical application of the simplified strong ion acid-base model involves calculation of the strong ion gap instead of the anion gap in order to detect the presence of unmeasured strong anions in plasma.¹² Recently we found that a hyperphosphataemic acidosis is also frequently evident in dehydrated neonatal diarrhoeic calves and accounts in addition to D-lactate in large parts for the increase of the unmeasured plasma anion concentration, quantified by calculating the anion gap or strong ion gap. It is, however, likely that still unidentified anions like uremic anions are present in severely dehydrated calves which significantly contribute to the development of acidaemia.¹³

Irrespective of the cause of metabolic acidosis, intravenous treatment with sodium bicarbonate is the method of choice for diarrhoeic calves

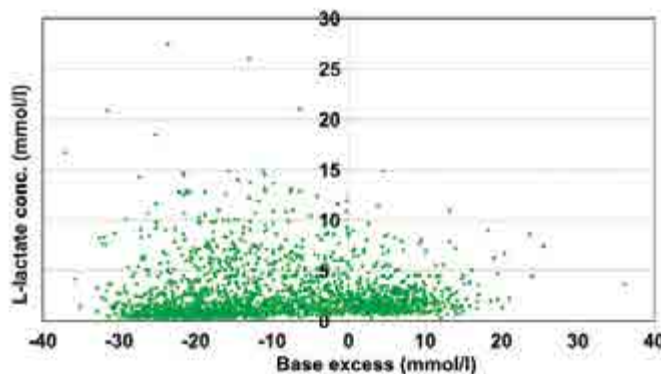


Figure 1: L-lactate concentration and base excess in 2019 calves with neonatal diarrhoea

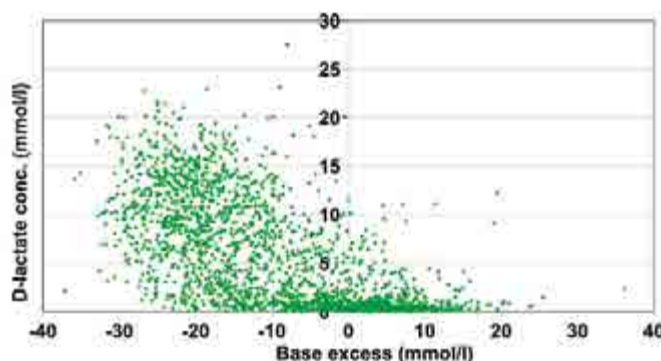


Figure 2: D-lactate concentration and base excess in 2019 calves with neonatal diarrhoea

that are considerably acidotic. The concentration and volume used can be varied according to the degree of dehydration. The amount of sodium bicarbonate needed for correction of acidosis can be estimated according to the clinical signs detected¹⁴ (Table 1).

Table 1: Estimation of the degree of acidosis in diarrhoeic calves with the help of clinical signs.

Clinical signs	Degree of acidosis	Therapy/required amount of sodium bicarbonate
Alert, standing, no dehydration	No acidosis	oral electrolyte solutions
Standing securely, enophthalmos	mild (BD about 10 mmol/l)	250 mmol (20 g)
Tired or listless, standing wobbly, has to be helped to stand up	moderate (BD about 20 mmol/l)	500 mmol (40 g)
Apathetic to comatose, sternal or lateral recumbency or barely able to stand and impaired palpebral reflex	severe (BD over 20 mmol/l)	750 mmol (60 g)

D-Lactataemia

Until the 1980s, D-lactic metabolic acidosis was only known to occur in adult ruminants due to acute ruminal acidosis after carbohydrate overload.¹⁵ In calves the production and accumulation of D-lactate was

first described in the context of ruminal drinking. After it has been known for a while that the accumulation of milk or other solutions containing readily fermentable carbohydrates predominantly leads to lactic fermentation in calves, it could be shown that continuous force-feeding of milk not only led to accumulation of D-lactic acid in the rumen, but also to D-lactataemia.^{16A} It was only after the development of stereospecific assays^{17,18} for the measurement of D-lactate hugely facilitated research in the area, that its importance in the context of neonatal calf diarrhoea was discovered.

Carbohydrates that escape digestion and absorption in the small intestine due to enteritis represent a rich fermentative substrate for colonic bacteria growing in the large intestine. The subsequent acidification of the intestinal milieu favours lactic acid producers, resulting in generation of larger amounts of L- and D-lactic acids. Multiple studies indicate the intestines as source of D-lactate in diarrhoeic calves. Researchers found elevated D-lactate concentration in the faeces of diarrhoeic calves when compared to healthy controls, and/or could demonstrate D-lactic metabolic acidosis in diarrhoeic without elevation of D-lactate concentrations in the rumen.^{6,8,19-21}

In calves with neonatal diarrhoea the risk to develop D-lactic metabolic acidosis is age dependent.²² In general calves that suffer from diarrhoea within their first 5 days of life have significantly higher base excess and significantly lower D-lactate concentrations than older calves (Figure 3). Koch and Kaske²³ described that calves infected with enterotoxigenic *Escherichia coli* were younger and less acidaemic than calves suffering from neonatal diarrhoea caused by viral or cryptosporidium infections. This is compatible with the hypothesis that an increase in production of D-lactate in diarrhoeic calves results from villous atrophy in the small intestine with maldigestion and malabsorption and subsequent microbial fermentation of substrates in the large intestine.⁶ In a study investigating D-lactate concentrations throughout the gastrointestinal tract of calves that have been euthanized for various medical reasons, elevated D-lactate concentrations were found throughout the entire gastrointestinal tract in some calves. In these calves significantly more Gram-positive rods could be found in the abomasum, the distal part of the jejunum, the caecum and the colon when D-lactate was present (>3 mmol/L), compared to cases with negligible concentrations of D-lactate at the respective locations. At present, it can only be speculated as to

whether D-lactate is actually produced throughout the whole intestinal tract, or only in circumscribed areas and subsequently distributed by means of intestinal motility.²⁴

The serum D-lactate concentration plays a major role in the development of the clinical picture in calves with diarrhoea. It has been shown in diarrhoeic calves that D-lactate concentration was associated with impaired posture, behaviour, and especially impairment of the palpebral reflex, whereas the sucking reflex appeared to be influenced by dehydration and metabolic acidosis.²⁵ Experimentally induced severe D-lactataemia without acidosis also resulted in profound changes in posture, behaviour, and palpebral reflex in healthy calves.²⁶ Induction of hyperchloraemic metabolic acidosis with a mean base deficit of up to 22.4 mmol/L with an IV infusion of 4000 mL of a solution containing 400 mmol HCL in 0.9% NaCl over a period of 80 min in healthy calves did not cause any central nervous system (CNS) signs.²⁷ In prolonged hydrochloric acid (HCl)-induced metabolic acidosis, however, a mild depression of neurological function could be observed. In this study acidosis was produced by infusion of either isomolar DL-lactic acid, L-lactic acid, or HCl, respectively, over a period of 6 h. Only DL-lactic acidosis was associated with severe disturbances in neurological functions (i.e. ataxia, and depressed menace, palpebral and tactile reflexes), whereas either form of acidosis triggered depression of the sucking reflex.²⁸

Even though the serum D-lactate concentration is helpful for the clinical assessment of the degree of metabolic acidosis there is no need to take it into special consideration for therapeutic measures, since D-lactate concentrations decrease regularly within 24 to 48 hours of initiation of therapy targeted at correction of dehydration and acidosis.²⁹ The mechanism that leads to normalisation of D-lactate values is not completely clear. Most likely it is caused by a combination of increased renal excretion triggered by enhancement of renal perfusion due to rehydration, and decreased production due to the correction of metabolic acidosis. It can be speculated that this happens due to alkalinisation of the intestinal lumen after the deficit of bicarbonate is corrected.³⁰

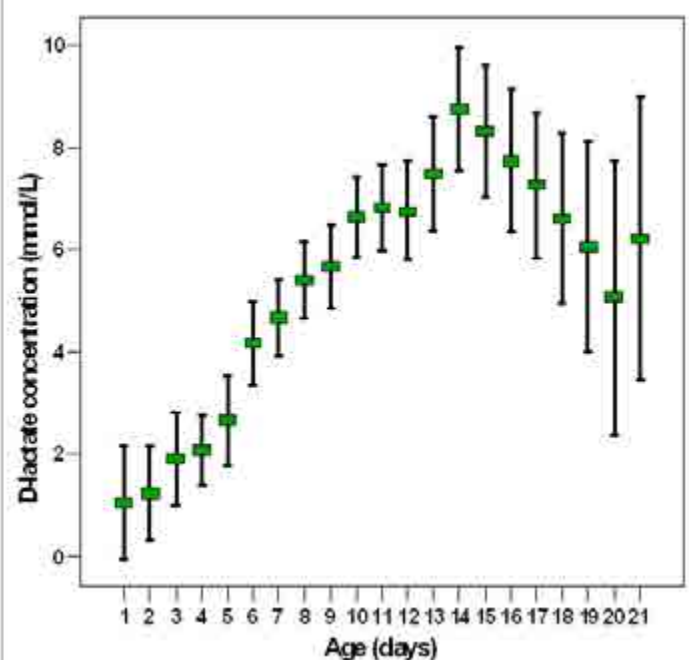
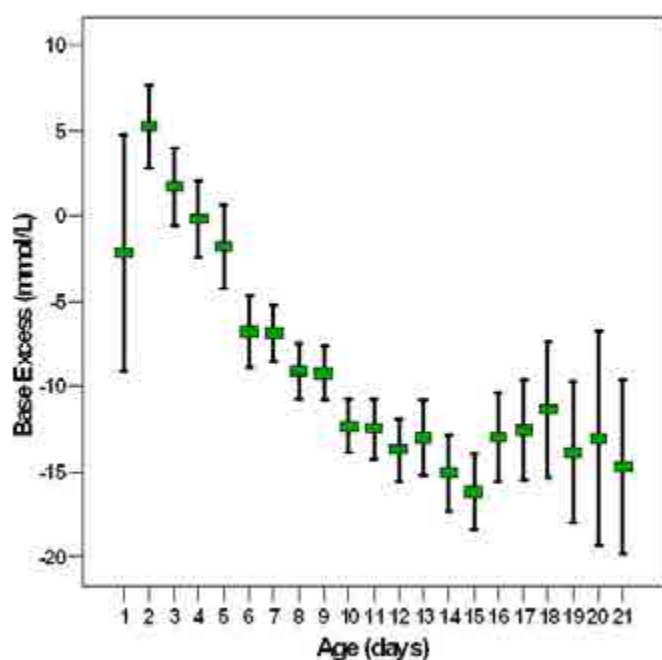


Figure 3: Base excess and serum D-lactate concentration (mean \pm 2SE) related to age in 2019 calves with diarrhoea upon admission.

Potassium balance disorders

Electrolyte imbalances are common in neonatal diarrhoeic calves and are closely linked to derangements of acid-base status. Although neonatal diarrhoeic calves have a clear negative potassium balance due to intestinal losses and low milk intake,³¹ data of a large study population of hospitalised calves indicate that they usually show normo- or even hyperkalaemic plasma concentrations.^{32F}

Hyperkalaemia is a clinically relevant electrolyte imbalance in those animals and has historically been attributed to an acidaemic state with intracellular buffering of hydrogen ions and impairment of the Na⁺/K⁺-ATPase on cell membranes with subsequent diminished intracellular translocation of potassium ions as the proposed underlying mechanisms.^{33,34} In classic publications, the response of plasma potassium concentration has been characterized by a linear increase of 0.3 to 0.6 mmol/L for every 0.1 unit reduction in blood pH.^{35,36} However recent studies indicate that the presence of a hyperkalaemic state in diarrhoeic calves strictly depends on the nature of an existing acidosis but not on acidaemia per se. Especially D-lactic acidosis is only rarely associated with increased plasma potassium concentrations and was identified as a protective factor for the development of hyperkalaemia. More importantly plasma potassium concentrations in diarrhoeic calves are most closely correlated to clinical and laboratory indices of dehydration, which indicates that a decrease of renal glomerular filtration rate plays a central role in the development of a hyperkalaemic state.^{32,37,37}

Clinical alterations of hyperkalaemia are related to an impaired neuromuscular excitability which is further exacerbated by the presence of hyponatraemia and metabolic acidosis,³⁸ conditions which are usually present in affected calves. Due to the potential cardiotoxicity (Figure 4), acute hyperkalaemia represents a potential life-threatening state and was traditionally considered to be an important cause of death in diarrhoeic calves.¹ Electrocardiographic manifestations typically include large and spiked T-waves, increased QRS-duration, flattened or missing P-waves and potentially R-R irregularities.^{33,39-41} However an ECG is rarely available in ambulatory practice, and there are also typical clinical signs that should alert the examiner to the presence of hyperkalaemia. In our experience, bradycardia or cardiac arrhythmias are rarely detectable and the latter had also a low sensitivity for the detection of a hyperkalaemic state in an observational study.³⁷ More reliable findings include severe clinical dehydration, cyanosis and especially impairments of ability stand.³⁷ The latter is likely related to skeletal muscle weakness which is also a prominent feature in hyperkalaemic human patients.⁴² Hyperkalaemia in diarrhoeic calves can therefore produce a clinical picture which is somehow comparable to marked D-lactic acidosis. However since a hyperkalaemic state in diarrhoeic calves is usually accompanied by normal or only slightly elevated plasma D-lactate concentrations, those animals usually exhibit a normal palpebral reflex, which allows to clinically distinguish between those two conditions.

Acute hyperkalaemia should be considered as an emergency and many affected calves are presented with signs of shock or in a somehow "labile" state. Stress needs to be avoided as much as possible and treatment objectives should focus on a rapid stabilization of the calf. In this respect, hypertonic infusion solutions such as 8.4% hypertonic

sodium bicarbonate or 7.2% NaCl have a sound physiological basis in the initial treatment of affected calves.⁴³ Those infusion solution induce a rapid plasma volume expansion, potentially enhance the redistribution of potassium ion into the cells, and have the ability for a rapid reversal of electrocardiographic manifestation.⁴³ However hypertonic saline might be inferior to sodium bicarbonate as it does not correct concurrent acidaemia which is usually present in those calves. A recent study showed, that intravenous administration of hypertonic sodium bicarbonate in a dosage of 250-500 ml indeed induces an immediate and sustained potassium lowering effect which was most closely correlated to an increase in venous blood pH.⁴⁴ In that study, a subsequent decrease of plasma potassium concentrations was further associated with changes of hydration status, such that we currently recommend to additionally infuse larger volumes of crystalloid infusion solutions. Glucose containing infusion solutions might have an additional beneficial effect in this respect due to an insulin-dependent transcellular shift of potassium ions.

As outlined above, hypokalaemia is a rare finding in neonatal diarrhoeic calves, especially if they are suffering from metabolic acidosis and/or dehydration. However, in a recent study the proportion of hypokalaemic calves after 24 hours of fluid therapy was reported to be twice as high as on admission to a veterinary teaching hospital. A hypokalaemic state was especially prevalent after 24 hours of therapy in calves which suffered from severe metabolic acidosis, had a low intake of milk and a long history of diarrhoea,⁴⁵ which is likely related to a depletion of body potassium stores. Hypokalaemia was not found to be associated with any clinical alterations in that study. However further studies are necessary if such diarrhoeic calves benefit from oral or parenteral administration of potassium salts.



Figure 4: Electrocardiographic findings in a hyperkalaemic neonatal diarrhoeic calf including arrhythmias and missing P-waves (plasma potassium concentration 9.4 mmol/L).

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Managing Liveweight Production From Beef Breeding Herds

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Introduction – asking the right question

Too often when the question is asked, 'How is your beef breeding herd going?' the answer is a performance measure such as the proportion of cows pregnant or calves weaned. However, profit is primarily a function of live weight production, its value, and the cost of production. Therefore, the question should be 'How many kilograms of live weight do you produce annually from this management group (herd) of breeding females per hectare?' For example, if the farmer puts 100 tonne of cows in a paddock after completing pregnancy diagnosis of the herd then 12 months later how many tonnes of beef have been harvested from the herd (includes calves weaned and any cows and bulls sold). The role of veterinarians consulting to beef breeding farms should be to develop management strategies to improve herd live-weight production and identify opportunities to reduce cost of production. However, beef cattle farmers typically use veterinarians only to conduct pregnancy diagnosis, breeding soundness examination of bulls, and investigate outbreaks of disease or lower than expected reproductive performance.

Measuring live weight production and fertility of beef herds

Annual pregnancy diagnosis and foetal aging, assessment of lactation status at branding and weaning, and weighing a representative sample of cows and weaned calves provide the data required to define liveweight production and fertility of breeding herds. Transrectal foetal ageing enables estimation of month of conception and calving, which when conducted in two consecutive years enables the interval from calving to conception to be estimated. Assessment of lactation status after the expected period of calving enables determination of the incidence of foetal and calf loss. Summarising the data in the form of predicted month of calving histograms informs decisions on when to conduct branding or weaning, and enables the veterinarian to identify potential causes of reduced performance (Figure 1).

Relatively simple paper-based recording systems have been developed by Fordyce et al. (2014a) that enable live weight production and performance of beef breeding herds to be monitored. However, adoption of these recording systems has been highly variable with many extensive rangeland farmers in particular, still unable to accurately count the number of females on their farm or the number of calves weaned annually. The development of electronic identification (EID) systems to enable crush/chute-side and remote electronic data capture can be effective in monitoring the production and performance of extensively managed beef cattle (McGowan et al. 2014a; Swain and Friend 2013).

Operating herd management software using a ruggedized laptop and an electronic enter pad connected to an EID reader mounted to the crush/chute and electronic scales mounted under the crush/chute enables capture of 12 to 20 pieces of data on cattle at typical processing rates of 60 to 100 animals per hour (McGowan et al. 2014). Currently, the major limitations of this system are loss of EID tags from cattle, estimated at about 8% within 3 years of insertion in a tropical rangeland environment (McGowan et al. 2014a), and the inability to complete data analysis on the day of data capture. Cloud-based systems will allow real-time assessment of individual management information through viewing and recording farm data from any location using many devices (eg, laptops, phones, tablets). This will also enable interconnectivity for external on-the-fly data analyses that could validate data plausibility and estimate individual and management group production and performance indices.

Predicting live weight production that can be sustainably achieved by breeding cattle

Beef breeding herd management varies considerably across the world, but the fundamental principles remain constant. A key principle is to know what level of live weight production a specific feed resource, usually native or improved pasture, is capable of sustaining. In a large study of factors affecting the reproductive performance of commercial beef breeding herds in northern Australia (McGowan et al. 2014a) farmers/managers were asked estimate average annual growth of yearling steers if they grazed the pastures grazed by the heifers and cows enrolled in the study. Mean annual steer growth varied from 100 to 200kg associated with large differences in soil fertility and vegetation type. One easily measured estimate of live-weight production from breeding herds/management groups is weaner production (kg/cow = total weight of calves weaned / the number of cows retained for calving and then mating in the next year). McGowan et al. (2014a) demonstrated that commercial weaner production was on average equivalent to annual estimated steer growth. Research is currently being conducted in northern Australia to further investigate whether routine grazing of

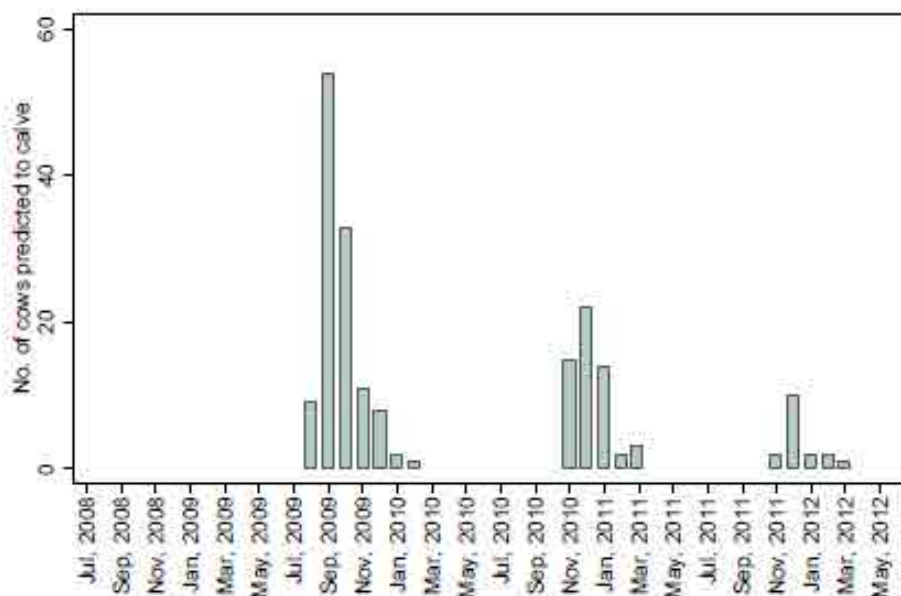


Figure 1. Calving pattern for a group of heifers that were monitored for 3 years in a herd which had a defined mating period. Note the shift to the right in month of calving which reduces the opportunity for late calving females to reconceive.

a sample of representative yearling steers in each paddock grazed by cows and heifers can be used to estimate the expected average weaner production from these paddocks. Clearly, because of the often marked variation in seasonal weather conditions this approach will need to be conducted over at least three years to obtain a reasonable estimate of average weaner production.

Beef farmers often aim to achieve a certain average weaning weight which may not take into account the feed resource(s) they have available for their cattle. For example, if the annual estimated growth of steers in a particular situation is 100kg and the average weight of calves weaned is 200kg, where does the additional 100kg come from? Clearly, it has to come from mobilisation of fat and protein reserves from the heifer or cows that produced these calves. In this low annual growth environment, these cows may lose 100kg during lactation (2 body condition scores - 1-5 scale), and as a consequence are unlikely to reconceive and have an increased risk of mortality. The farmer may then be forced into supplementary feeding to prevent cow mortalities; but then the question must be, 'is this intervention likely to be profitable'?

Farmers often want to know what level of production they should be aiming for. Although benchmarking herd or management group production causes angst amongst some economists, this is entirely valid if referenced against a measure of what the specific feed resource is capable of producing. An example of this approach is provided in Table 1.

Table 1: Annual weaner production from management groups of cows in northern Australia against estimated annual average steer growth (McGowan et al. 2014a)

Mean annual steer growth (kg)*	No. of herds	Weaner production (kg/cow)		
		25 th percentile	Median	75 th percentile
200	33	164.0	191.0	240.0
180	33	160.7	194.6	220.1
170	29	134.9	163.0	182.6
100	59	74.0	93.3	112.4

*Estimated growth of steers grazing same pasture as cows

What are the major drivers of live weight production of a breeding herd

The concept of live weight production (McGowan et al. 2014a) is that over a one-year production cycle, a cow's production, if she remains alive, is the sum of her live weight change, and the weight of any weaned calf. The business makes money by selling the annual live weight produced either directly, or after transfer to another sector of the herd for value adding. This is equivalent to how any business measures productivity.

McGowan et al. (2014a) demonstrated that no single performance measure of a population of commercial beef breeding herds in northern Australia was predictive of annual live weight production. However, this is not surprising since no measure of fertility takes into account heifer/cow mortality or annual change in live weight. Using annual live weight production as a primary measure of "how a herd is going" encourages a more holistic approach to herd management. However, regardless of whether beef breeding herds are control mated or mated continuously, key drivers of live weight production are the percentage of lactating cows pregnant within 4 months of calving (estimates the proportion of cows likely to wean a calf in consecutive years), the annual total percentage of pregnant cows, the percentage of foetal and calf loss between confirmed pregnancy and weaning, average weight of weaned calves, live weight change of heifers/cows and percentage heifer/cow mortality.

Developing management strategies to increase live weight production and reduce cost of production

It does not matter whether you are consulting to breeding herds of 5 cows or 150,000 cows, the critical influence of nutrition on reproductive performance is the same. Too often undue emphasis is placed on investigating infectious diseases and trace element deficiencies rather than focussing on body condition of heifers and cows in the last trimester of pregnancy and first 3 months of lactation. The major factors affecting the percentage of cows becoming pregnant within 4 months of calving and percentage of pregnant females failing to wean a calf in tropical rangelands typical of northern Australia have been described by McGowan et al. (2014b) and Fordyce et al. (2014b), and in many cases are remarkably similar to those identified as being important in more intensive temperate beef breeding regions of the world. The overall approach we recommend is after defining the likely factors affecting production and performance implement 'best practice' management strategies which are summarised as:

Manage the feed-base

You cannot make something from nothing. Cattle can only achieve net live weight production if energy and protein intake is above that required for maintenance. Beef breeding businesses are built on ready access to productive, palatable, nutritious pastures and good quality water. The principles of 'best practice' grazing management must be understood and implemented.

Key management practices

- Budgeting available feed to meet short and medium term cattle requirements
- Good grazing management to allow pasture recovery, eg, rotational grazing, or in tropical rangelands deliberate withholding of grazing of selected paddocks over the wet season
- Limit grazing distance from water to <2.5 km where possible
- Active pasture development and rehabilitation
- Fencing to control overutilization of preferred land types including riparian zones
- Use supplements that augment sound basic management. For example feeding supplemental phosphorous to late-pregnant heifers and first-lactation cows where risk of phosphorous deficiency is high. If good grazing management and lactation management practices are implemented then feeding of nitrogen supplements during the dry season in arid tropical rangelands may only be necessary during periods of severe drought.

Manage lactation

Cows have amazing capacity to meet their energy, protein and macro-mineral requirements from available pasture and mobilisation of their own body tissue reserves. However, where cattle draw down on their body tissue reserves (eg, during lactation) this must be followed by a period of re-alimentation in preparation for the next reproductive cycle. Thus the timing of weaning is critical because cows must have sufficient access to pasture of adequate quality to replenish body tissue reserves prior to the next calving event.

Key practices

Manage weaning to conserve body condition of cows in preference to achieving high live weight weaners, ie, the decision on timing of weaning should be made on the basis of cow body condition, not an average weaning weight target.

Use pregnancy diagnosis and foetal aging to segregate cattle for different nutritional management and efficient weaning. It is particularly important that heifers are managed as a discrete group until they are confirmed

pregnant after calving for the first time. Also in continuously-mated herds identification of heifers and cows likely to calve at a time when pasture quality and quantity is very limited is critical to minimising cow and calf mortalities.

Wherever possible mating should be controlled to ensure heifers and cows calve close to the time when the likelihood of significant improvement in seasonal pasture quantity and quality is high. Alternatively in continuously-mated herds use foetal aging to segregate cows into approximately 3-month calving periods which can be matched with feed available, handling and husbandry.

Manage cattle health & stress

This primarily involves implementation of evidence-based control strategies to prevent infectious causes of heifer/cow death (eg, clostridial diseases including botulism, babesiosis), clinical illness (eg, bovine ephemeral fever), subclinical disease (eg, external/internal parasites), and infectious causes of embryonic, foetal and calf loss (campylobacteriosis, trichomoniasis, bovine viral diarrhoea virus). Also breeding females and their offspring may be exposed to a wide range of environmental stressors which can severely impact on both survival risk of the calf and the dam.

Key practices

A risk-based approach to control of infectious diseases should be used involving assessment of the immune status of the dams including determination of whether the herd or management group is endemically infected, and risk of introduction of infection.

Provide protection from environmental extremes (floods, blizzards, heat wave), especially for young calves and their dams

Where possible, avoid handling calves less than one month of age

Manage breeding

Bull fertility and genetics have a profound effect on business outcomes and herd productivity. Frequently the 'low hanging fruit' in a beef breeding business is the bull percentage used. In a study of bull selection and management McGowan et al. (2014a) found that approximately three quarters of farmers or managers used above the recommended 2-2.5% bulls. Bull costs per calf born are an important cost eg, if the average cost of replacement bulls is \$4,000 and bulls are mated at 2% versus 4% then the annual costs per calf assuming a weaning rate of 80% are \$14 and \$27, respectively.

Key practices

- Establish a genetic improvement program to achieve long-term increases in fertility as well as improvements in traits such as carcass quality, and in harsh environments, adaptive traits
- Select replacement bulls that have passed a breeding soundness evaluation. Select physically-sound bulls with at least average scrotal circumference for breed and live weight, and greater than 70% normal sperm.
- Replacement bulls should be introduced to the farm at least 4 months prior to use. They should be vaccinated against known causes of death, illness and reproductive loss.
- Mate at no more than 2.5% sound bulls.
- Select bulls from dams that have weaned a calf from their first two mating opportunities.
- Bulls should be managed to ensure they maintain satisfactory body condition (at least BCS 2.5 - 1-5 scale). Treatments to control external and internal parasites are recommended as bulls generally carry higher burdens of both.
- Herd bulls should undergo at least a general physical examination and detailed examination of the external genitalia annually prior to

mating and bulls should be considered for culling when they reach 8-9 years of age.

Initiating adoption of management changes – how successful have we been?

In preparing this paper we are very conscious that David Mossman, Basil Lowman and Keith Entwistle beautifully described the approach to improving reproductive performance in a series of publications in the '70's and '80's. However, adoption by farmers and managers of many of their recommendations has been disappointingly slow; eg, McGowan et al. (2014a) reported that in northern Australia only about a quarter of farmers/managers routinely used a breeding soundness examination including microscopic examination of semen to select replacement bulls. As veterinary advisors to beef breeding herds we have to accept that in many cases we have failed to effectively communicate how and why producers should adopt recognised 'best practice' recommendations. In some cases we focus only on the potential positive benefits of our recommended changes to management without equally acknowledging the potential negative outcomes. A good example of this is where a farmer adopts your recommendation on lactation management which results in a significant increase in the proportion of the herd becoming pregnant within 4 months of calving and thus contributing a weaned calf each year. If the farmer does not adjust his/her culling and selling strategies then there is a significant risk of overgrazing and degradation of the pasture. Further, too often we assume that the terms we use are universally understood by farmers yet there is clear evidence that in many cases there is considerable confusion amongst farmers, advisors and veterinarians; eg, the definition of weaning rate is highly variable.

Take home messages

- Understand what live weight production your client's beef breeding herd's feed resource is capable of sustainably supporting.
- Measure the actual production and performance of each breeding management group or herd you consult to.
- Understand the key drivers of live weight production from beef breeding herds.
- Understand how to cost effectively control the major factors affecting these drivers of live weight production

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Bovine abortion/stillbirth investigation: a practitioner-focused approach

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Introduction

Abortion and stillbirth diagnosis rates do not appear to have improved over time internationally despite the development of molecular diagnostic techniques and the discovery of new abortifacients. While reasons for the overall low diagnostic rates from fetal investigations are detailed hereunder, this paper addresses this challenging conundrum with a view to practitioner-led improvements in diagnostic rates.

'Observed abortion'

From a practitioner and their clients' perspectives, abortion may be defined as the expulsion of a non-independently-viable fetus before full-term that is likely to be observed (and possibly submitted for examination) by a farmer, i.e. an 'observed abortion'. Irish data suggest the period of risk for an observed abortion is from 120 to 260 days of gestation (Mee and Sanchez-Miguel, 2015). The fetus at 120 days is the size of a small cat with a crown rump length (CRL) of 20-30cm and weighs ~1kg.

an abortion can be defined for practical purposes as the expulsion of a visible, but non-viable, fetus

The majority of laboratory-investigated abortion cases are from the last trimester (Table 1), while the majority of non-submitted abortions are from the first two trimesters; this leads to under-estimation of real abortion rates (Thurmond et al., 1994). For example, over 95% of aborted foetuses submitted to an Irish regional veterinary laboratory were from the fourth month of pregnancy on (>120 days), (Table 1).

Table 1. Gestational age of laboratory-investigated aborted foetuses over a 25-year period.

Trimester	Month of gestation	Fetuses (No.)	%
First (early)	2	73	0.7
	3	298	2.9
Second (mid)	4	841	8.2
	5	1393	13.7
	6	2069	20.3
Third (late)	7	2858	28.0
	8	2661	26.1

Source: Cork RVL, 10,193 fetuses (1980-2003)

'Stillbirth'

Perinatal mortality may be defined as death of the fetus or perinate before, during or within 48 hours of calving at full term (≥ 260 days) (Mee et al., 2014). While 'stillbirth' is commonly used as a synonym for perinatal mortality it may be defined as the birth of a fetus before or during calving at full term. These gestational and perinate age thresholds are arbitrary and vary both intra- and inter-nationally.

REPORTING OF FETAL/PERINATAL MORTALITY

Before addressing the titular topic; investigation, the issue of reporting needs to be addressed as it is equally if not more important.

Under-reporting

Under-reporting is a major issue with bovine fetal/perinatal mortality internationally. To some risk-averse clients the loss of a single valuable pregnancy may trigger a veterinary investigation (threshold for investigation) while for the majority of clients multiple losses need to occur before this threshold is exceeded. A few abortions in large herds are 'expected', i.e. abortion is considered a 'normal' event as long as it is sporadic. In a French study only 20% of beef farmers who had an abortion or stillbirth reported it (Bronner et al., 2013). A fetal mortality rate of $\geq 5\%$ is the investigation threshold used by the majority of veterinary respondents in the EU (Bronner et al., 2014). However, a cluster of cases is a more important 'tipping point' for investigation in seasonal calving herds, than % of pregnancies aborted.

a cluster of abortions is a more important tipping point for investigation than % of pregnancies

Proximity to the laboratory, number of losses, 'farm blindness' and lack of understanding influence the likelihood of sample submission.

UNDER- AND OVER-TRIAGE

The approach to a sporadic abortion/stillbirth in a herd without a history of problems will differ from that in a herd with a series of cases. This is referred to as under-triage, whereby such sporadic cases are not investigated as thoroughly as cases from multiple mortality herds. However, over-triage may be warranted even for sporadic cases on farms a long distance from the local laboratory as resubmission of material is less likely. One cannot predict that the index abortion case is not the first of many in a herd outbreak.

all abortion 'storms' start with a single case

Studies have shown that of abortions only approximately 20 to 30% are observed (Gadicke and Monti, 2013) suggesting that in many cases the first case presented (index case) is not in fact the first case that occurred, merely the first that was observed.

PROBLEM HERDS

The herd-level occurrence of abortions/stillbirths follows a right-skewed distribution with most herds having no or low losses but a minority of herds have high losses. The threshold at which loss prevalence becomes a 'problem/cluster/storm', and hence is investigated, is usually defined by the herdowner. Norm-referenced thresholds are cohort-based which may reflect the national recorded abortion rate. Criteria-referenced thresholds are based on metrics other than those based on cohorts, e.g. a theoretical or empirical threshold. While the latter has traditionally been 2% for abortions, it is suggested a higher figure may be more appropriate to include non-observed abortions (Mee and Sanchez-Miguel, 2015).

VETERINARY PRACTITIONER-LED ABORTION/STILLBIRTH INVESTIGATION

When approached by a client with an aborted cow or a stillbirth problem (as defined above) the following standard operating procedure (SOP) is recommended.

SOP FOR ABORTION/STILLBIRTH INVESTIGATION

This involves three simple steps; collect a history, examine pregnant animals and examine the foetus/placenta.

1. Collect a history

First establish the nature and extent of the problem by investigating possible risk factors associated with the loss/es. Standard questions to ask include how many cows have aborted/had stillbirths, how many cows are pregnant, what fetoplacental material is available, is the dam sick, are most losses in heifers, and recent husbandry changes. A proforma questionnaire is used by veterinary laboratory staff to collect the history

prior to the postmortem examination; practitioners might consider using such a format for problem herds. In addition to collecting information on the individual abortion/stillbirth, the herd health history is important. For example, last vaccinations against fetopathogens, recent cattle purchases and bulk milk test (BMT) test results.

2. Examine/sample the pregnant and aborted/stillbirth animals

The pregnant cohort

Examining the pregnant animals and their environment allows the practitioner to assess their general health, body condition score (BCS) and feeding management. Cohort sampling of dams in the affected group can be useful in determining micro/macronutrient status and differences in exposure (presence/absence, prevalence) between affected and unaffected dams (case-control); but vaccinal status needs to be known.

The dam

Clinical examination of the dam may reveal pyrexia, diarrhoea, respiratory signs, etc. A faecal sample may be useful where salmonellosis is suspected. Single blood samples from aborted cows are the most common samples collected to investigate bovine abortion (sero-diagnosis). These are moderately useful as proxy samples for fetal material. For example, a single blood sample from the dam of an aborted fetus can be up to 85% accurate (non-vaccinated cows) in predicting a fetal culture-positive result (e.g. Salmonella Dublin), (Sánchez-Miguel et al., 2011). However, the primary value of maternal serum is as an exclusionary test for maternal antibody, i.e. a negative result rules out some causes, e.g. Neospora caninum. a single dam blood sample is 85% accurate in diagnosing S. Dublin abortion

Paired sera (more than two weeks apart) may detect rising titres (two to four-fold) for some abortifacients (e.g. S. Dublin SAT) but not for others (e.g. Leptospira spp. Neospora) due to the lag phase between infection and fetal mortality. In vaccinated herds natural infection can still be distinguished where DIVA vaccines are used (gE-deleted BHV) and where titres are much higher than those expected from vaccination (e.g. Leptospira) this suggests current, active infection.

**3. Examine the foetus and placenta
ABORTION/STILLBIRTH FETOPSY SOP**

As with a clinical examination on a live animal a systematic approach means that important lesions are not missed due to focusing on the obvious lesion/s; this involves three steps - external and internal examination and sampling the carcass.

1. External examination

The external examination will confirm the animal identification, fetal preservation, maturity, size for date/plurality, and detect defects, discharges and predation. The stage of development of the foetus may be estimated from its body weight, size (e.g. crown-rump length; CRL), (Table 2) and external developmental indicators (dentition, pilosity).

Table 2. Physical characteristics of aborted foetuses at different months of pregnancy

Month of gestation	Body weight (kg)	sCRL* (cm)
6	5	50
7	15	70
8	30	90
9	40	95

*sCRL = straight crown-rump length.

Gestational age may be estimated from sCRL as follows: gestational age (days) = 68 + 2.25 x CRL (cm)

2. Internal examination

An illustrated guide to bovine fetopsy by the practitioner has recently been published (Mee, 2016).

3. Sampling the carcass

A sample selection algorithm for aborted/stillborn fetuses is outlined in Figure 1. It is not possible to be prescriptive about test selection as laboratories differ in the test menu and tiers they offer. Test selection will also be determined by the anamnesis, degree of carcass autolysis and costs. Rather than 'necropsy by algorithm' the decision-making of the pathologist still determines whether to collect samples. Additionally, samples can be discarded if collected in the early stages of the necropsy where subsequent examination reveals the likely cause does not require laboratory testing.

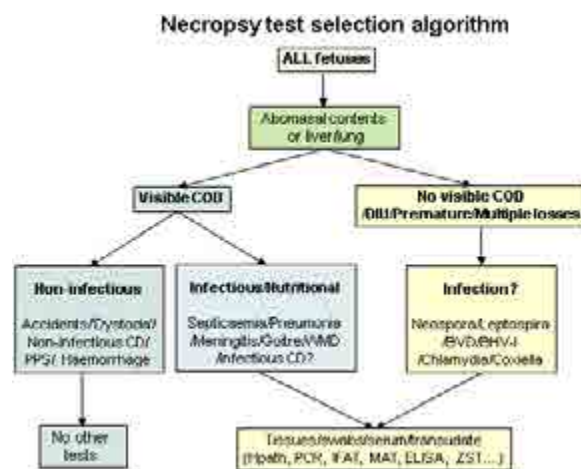


Figure 1: Practitioner-focused necropsy sample selection decision tree for aborted/stillborn foetuses (COD=cause-of-death, CD=congenital defect, DIU=dead in utero)

Microbiology samples

Abomasal contents can be sampled aseptically by searing the abomasal serosa with a heated scalpel blade and aspirating a sample into a plain vacutainer tube. In general, tissue samples are preferable to swabbed samples and surface swabbed samples are preferable to fluid swab samples. If an abomasal sample is unobtainable (due to scavenging) or if septicaemia is suspected, lung, liver or brain samples are suitable alternatives. Brain sampling is of particular value in scavenged and mummified foetuses.

Serology samples

Serological sampling can be useful after the first trimester (>120 days) when the foetus is immunocompetent as antibodies indicate fetal infection (but not necessarily fetopathy, e.g. Neospora congenital infection), assuming placental competence.

Trace element samples

Examination of the thyroid for goitre and submission of a fresh (I₂ content) and formalinised lobe (histopathology) will detect possible iodine deficiency. Where selenium deficiency is suspected a sample of the liver or kidneys should be submitted.

Histopathology samples

Samples for histopathology should include normal and abnormal tissue and should not be greater than 1 cm thick and 2 cm long. Many pathologists routinely sample lung, liver, thyroid, heart and brain. As the brain is of particular value in the histopathological diagnosis of neosporosis, submission of the skull or entire brain is recommended.

Table 3. Standard and additional samples to collect from aborted and stillborn fetuses*

For investigation of	Standard samples	Ancillary samples	Comments
Fetopathogenic bacteria and fungi (e.g. <i>Aspergillus</i> spp., <i>B. licheniformis</i> , <i>L. monocytogenes</i> , <i>T. pyogenes</i> , <i>S. dublin</i>)	Fetal stomach contents (FSC). Placenta	Fetal lung, liver, gall bladder, kidney, brain, eyelid. Dam vaginal swab, placentome, blood.	Ancillary samples where FSC/placenta unavailable/contaminated.
<i>Neospora caninum</i>	Fetal brain, serum/transudate	Fetal heart. Placenta. Dam/cohort bloods	Fresh brain/placenta for PCR, fixed brain/heart/placenta for histopath
<i>Leptospira Hardjo</i>	Fetal kidney, serum	Dam/cohort bloods	Fetal sample dependent upon lab. tests
BVDv	Fetal ear, spleen, thymus, serum	Fetal kidney. Dam/cohort bloods	Fetal sample dependent upon lab. tests
BHV-1	Fetal liver, serum.	Fetal kidney. Placenta. Dam/cohort bloods	Fetal PCR/histopath preferred tests
Micronutrient deficiencies	Fetal thyroid, liver, heart, rib	Dam/cohort bloods	Thyroid for iodine assay/histopath; liver for selenium assay, heart for histopath; bone for manganese
Gross lesions (e.g. fetal pneumonia)	Affected fetal organ	As required	As appropriate (e.g. bacteriology, histopath)
Genetic congenital defect	Fetal muscle	Dam hair follicles	Test for infectious teratogens also (e.g. BVDv, SBV)

*Standard and ancillary testing protocols are dependent upon local lab SOPs. Bacteriology/mycology (culture, stains, wet preps) and serology are generally routine tests for sporadic cases while other tests (e.g. histopath, PCR, FAT, IHC, micronutrient, DNA assay) can be added for multiple losses or at the discretion of the pathologist. Maternal vaccinal status affects use and choice of serology tests.

Standard abortion package

Be guided by your local laboratory about which samples they prefer to use routinely for each test ('standard abortion package') and which tests are non-routine (e.g. PCR, histopathology). While for sporadic cases the basic sampling package may suffice (Table 3), in abortion storms, tiered sample escalation may be advised as it provides the option of sampling, storing and testing as deemed necessary. Increasingly, genomic tests are being developed for developmental disorders which require specialist sampling, e.g. hair follicles.

Photo-documentation

Photo-documentation can be useful for inexperienced practitioners and for unusual lesions. In future practitioners may be able to use digital imaging technologies to enhance their PME yield, as currently occurs on North American feedlots (remote digital PME).

Sample submission

If samples are collected on a day when the laboratory is closed store the samples for culture, serology and histopathology in a fridge (4°C) and those for PCR in a freezer (-20°C). Practitioners need to be cognisant of postal regulations pertaining to packaging and labelling for biological substances. To facilitate processing of your samples in the receiving laboratory, complete their submission sheet and submit with the samples.

Examining the placenta

If the placenta has not yet been expelled at examination, collect a sample of the retained placentomes or a vaginal swab. A normal placenta at term weighs approximately 5kg, has 75-125 red cotyledons and has thin, translucent intercotyledonary tissue that sometimes contains adventitious placentation. Placentitis may manifest as discoloured, necrotic, exudative cotyledons and opaque, erythematous, thickened, oedematous intercotyledonary tissue. *submit three abnormal cotyledons/inter-cotyledonary tissue samples*

Ideally three abnormal cotyledons/inter-cotyledonary tissue samples should be submitted (preferably macroscopically abnormal and uncontaminated) for histopathological and microbiological examination (Gram smear, MZN, culture, PCR).

CAUSE-OF-DEATH

Diagnosis rates in aborted and stillborn fetuses are generally between 20 to 50% (Wheelhouse et al., 2015) and 30 to 75%, (Mee, 2015), respectively. The more carcasses that are examined the higher the herd diagnosis rate. In aborted fetuses the causes of death are often attributed to events which occurred days or weeks before foetal death so few cause of death lesions will be grossly visible; microbiological sampling is more important. However, in stillborn calves the cause of death is more likely to be periparturient-related, hence gross examination can often reveal more than laboratory testing (Mee et al., 2013). Foetopathogenic infections (Table. 4) and dystocia (abnormal calving) continue to be the most important diagnosable causes of bovine abortion (Clothier and Anderson, 2106) and stillbirth (Mee, 2015), respectively. Co-mortality is not uncommon in stillbirths and should be considered.

Table 4. Detection of infectious abortifacients in Irish cattle (DAFM, 2014)

Organism	Fetuses (%)
<i>Leptospira Hardjo</i>	7.5*
<i>Trueperella pyogenes</i>	6.1
<i>Salmonella dublin</i>	6.0
<i>Bacillus licheniformis</i>	3.6
<i>Neospora caninum</i>	3.5*
<i>Listeria monocytogenes</i>	2.6
<i>Aspergillus</i> spp	0.8
Other bacterial and fungal spp.	17.5
BVDv and BHV	NR
No agent identified	52.4**

NR=not recorded; *not all fetuses are tested for Lepto (fetal ELISA) and Neospora (fetal ELISA +/- histology); ** estimated as not all samples tested for Lepto and Neospora; Anon. (2015)

Diagnosis not reached (DNR)

The common reasons for DNR are diagnoses not attainable from PME, poor PME technique, insufficient, poor quality or incorrect sample submission and inadequate laboratory tests. In the case of aborted fetuses and 'unexplained stillbirths' non-infectious and non-dystocial causes are likely to be a major reason for DNR. While DNR may appear to a client or a practitioner as a failure of PME, it can be viewed as a successful rule-out of common causes of foetopathy.

TIME-OF-DEATH

Fetuses which die before birth will have varying degrees of autolysis; corneal opacity, excess body cavity transudate, decomposed organs (especially kidneys, liver, spleen and brain), haemoglobin imbibition, flaccid musculature, subserosal and subcutaneous serosanguinous oedema/emphysema and atelectatic lungs. Autolytic lesions develop within approximately 12 hours of death in utero and become more advanced over time. Fetuses which die during birth will not have signs of autolysis and may have signs of breathing (partial lung inflation), bradytocia (localised peripheral oedema) or traumotocia (trauma lesions with haemorrhage). Perinates which die shortly after calving will not have signs of autolysis and will have signs of breathing (partial or complete lung inflation, tracheal froth, umbilical thrombi), possibly attempting to stand or standing (worn eponychia, especially hind) and consuming colostrum (colostrum in abomasum).

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An epidemiological approach to herd problem-solving

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a. Investigating individual cases

'Making a diagnosis' is a central tenet of clinical veterinary medicine, allowing veterinarians to recognise a disease, and to assign this disease label to a case that presents with particular clinical or pathological characteristics. The diagnostic process draws on the education of veterinarians, but also their experience and judgement. Ultimately, assigning a diagnosis allows for ease of communication with colleagues along with the selection of appropriate and effective therapeutic actions.

Radostits et al. (2000) describe a number of different methods used by veterinarians when making a diagnosis on an individual animal, with the two most common generally being:

Pattern recognition, that is, recognition of the disease syndrome, after comparison with previous cases. This approach is common among experienced clinicians

Hypothetico-deductive reasoning. Initially, multiple plausible diagnostic hypotheses (differential diagnoses) are generated, based on presenting clues. Then, the clinician asks questions and conducts clinical examinations, to test (support or discount) each hypothesis. The process of hypothesis and deduction continues until one hypothesis is preferred over all others.

These approaches are used, often unconsciously, in combination or interchangeably depending on the clinical circumstances and prior experience.

A further more time-consuming approach is 'the key abnormality method', a five step process including sequential determination of:

- The abnormality of function present,
- The system or body as a whole or organ affected,
- The location of the lesion within the affected system or organ,
- The type of lesion, and
- The specific cause of the lesion.

b. Moving from the individual to the herd

As with individual animal medicine, 'making a diagnosis' is also a central tenet of herd health investigations. A correct herd-level diagnosis is generally needed, before proceeding to recommendations for appropriate herd-level strategies for control and prevention.

It is common, and understandable, that clinicians use familiar diagnostic methods during herd health visits; essentially, the same diagnostic method for herds as they would normally do for individuals. Using the two methods most commonly applied to individuals, there would generally translate into the following herd-level approaches to diagnosis:

Comparison with best-practice. Using this approach, the investigation includes a detailed review of farm practices relevant to this case, with the clinician (perhaps subconsciously) comparing practices on the affected farm with what they would view as best-practice. This can be thought of as a herd-level adaptation of 'pattern recognition', where deficiencies in comparison to 'good' farms form the diagnosis, e.g. mastitis problem due to suboptimal post milking teat disinfection. Remedying these deficiencies often forms the basis of action for improvement, e.g. ensuring all steps of the 10 point plan for mastitis control are in place.

Differential diagnoses, being a herd-level adaptation of 'hypothetico-deductive reasoning'. Using this approach, a series of plausible differential diagnoses are generated, typically based on initial

questioning, clinical examination of problem animals to give herd-level clues, to then perform hypothetico-deductive reasoning. Often this requires the use of additional diagnostic testing to identify the single most-likely hypothesis, e.g. investigating a problem of calf diarrhoea to identify the causal pathogen. This approach is then used to employ known efficacious controls relevant to the diagnosis.

These two approaches either in isolation or in conjunction, may often be used effectively to investigate herd problems. However, there are some problems with this direct translation of these diagnostic approaches. Farms are complex places, certainly a collection of individuals, but overlaid with additional layers of environment, management, feeding etc. There are particular challenges associated with each of the two suggested methodologies:

Comparison with best practice. This approach relies on a comprehensive understanding of all of the farm activities, which can be very time-consuming. It is important to recognise that perception of best-practice with vary between clinicians, influenced by many factors including clinical experience. Logically, therefore, lists for improvement (based on perceived differences between the affected farm and best-practice) will also vary between clinicians. Further, using this approach in isolation, it is challenging to prioritise the list for action, i.e. to identify those actions that, if addressed, would make the greatest difference with respect to the problem under investigation. This is especially true if the list is extensive, and can often lead to "fix everything" recommendations.

Differential diagnoses. This approach is of greatest use in simple cases, with a single disease process. However, an evaluation of differential diagnoses is of limited assistance in complex cases, e.g. those with multiple overlapping disease processes (Radostits et al., 2000) or where multiple pathogens, some possibly incidental, are identified during diagnostic testing.

c. An epidemiological approach to herd problem-solving

In many ways, epidemiology is quite unlike many other veterinary disciplines. In the traditional disciplines that we are most familiar, such as physiology, microbiology or pathology for example, the focus is at the level of the individual, the organ or the cell, seeking a clear understanding of processes of normality or disease. The key question here is 'how', to help us to better understand the 'why'? In contrast, epidemiology focuses on populations, underpinned by principles and utilising methods that seek to allow us to be solid conclusions from apparently uncontrolled situations. Here, the key questions are 'where', 'when' and 'whether', providing perspectives on the 'why' to complement those provided by the traditional disciplines. As such, epidemiology is a science that is well suited to herd investigations.

In a herd investigation, we generally focus on one or more of the following three questions:

- The cause, including what are the key points in the causal pathway that has resulted in the problem?
- Control, what can be done to stop it? What potential actions can be taken to effectively break the causal pathway?
- Prevention, what can be done to prevent it happening again? What actions can either remove causal factors or reduce the frequency or impact of their occurrence?

The issue of cause is particularly important in a herd investigation, noting that there is rarely a single cause; rather our interest will generally focus on causal pathways, on drivers and on risk factors. Herd problem-solving is typically conducted where performance is substantially less than the farm goals that were set.

Before proposing an epidemiological approach to herd investigations, it is critical to first distinguish performance and activity:

Performance refers to the output, or what is achieved. With respect to

milk quality, this could relate to the bulk milk somatic cell count achieved during a defined period of time

In contrast, activity refers to processes, or what was done. With respect to milk quality, this could include in-parlour practices such as stripping, teat dipping and cow segregation, the blanket use of dry cow therapy, the regular servicing of the milking machine etc

The epidemiological approach to herd investigation builds on the previously outlined strategies by introducing relevant epidemiological principles and methods. It seeks to overcome the challenges, as highlighted previously, when using diagnostic methods such as 'comparison with best-practice' or 'differential diagnoses'. The approach provides a structured and generic investigative framework, allowing clinicians to focus their efforts as quickly as possible during the investigation.

Step 1: Focusing on performance

During this initial stage of the investigation, it is critical to focus on performance, rather than activity. Indeed, any thoughts or concerns regarding activities on the farm should initially be ignored.

Firstly, the problem needs to be framed in terms of performance, for example, 'an increase in calf mortality', 'an increase in SCC', or 'an increase in the incidence of lameness'.

Then, a case definition is developed to enable cases to be identified and counted (for example, 'a calf that died during the last month', 'a cow with at least one SCC reading greater in 200,000 cells/mL during the current lactation', 'a cow with a lameness score of 3 or greater'). The case definition is very important, as it enables the investigator to count cases, and to distinguish cases from non-case (or control) animals. A numeric focus such as this is not a surprise, noting that epidemiology is a science underpinned by numbers.

Finally, these data allow a clinician to calculate simple measures of case frequency and of epidemiological association. With this information, it is possible to determine whether patterns (systematic differences between cases and controls) may be present, either in time, in space and between different animal groups.

If patterns are present (for example, 'a seasonal pattern of presentation is apparent', or 'there appears to be an increased risk among first lactation animals as compared with older cows'), these can be considered as clues, providing a sound basis for the development of multiple plausible causal pathways. In other words, these clues are used, where feasible, to rule in plausible hypotheses, and to rule out hypotheses that do not feasibly match the patterns observed.

Step 2: Investigating relevant farm activities and examining case animals

During step 2, the clinician only considers those activities that have the potential to plausibly contribute to any of the hypotheses identified in step 1. In other words, step 1 assists greatly, allowing considerable focus during the on-farm investigations. This is in contrast to the 'comparison with best-practice' method mentioned previously, where all aspects of herd activities need to be scrutinised.

During this step, a process of hypothetico-deductive reasoning is used, as outlined previously. Guided by the hypotheses developed during step 1, the clinician identifies relevant farm activities and seeks further information about each, through questions and by other means. In addition, clinical examinations and sample collection may be conducted, generally restricted to case animals, to test (support or discount) each hypothesis. The process of hypothesis and deduction continues until one hypothesis is preferred over all others.

In further contrast to the 'comparison of best-practice' method, using an epidemiological approach to herd problem-solving can assist in prioritising actions, specifically those actions that are likely to make the greatest difference, if enacted.

Step 3: Developing recommendations, and communicating these to farmers

Ultimately, for clinicians it is critical to be seen to add value beyond generic disease control practices. With individual animal cases, the treatments and solutions often lay in the hands of the clinician. In contrast, the resolution of most herd issues resides with the actions of the client. Therefore to be an effective herd clinician, communication and facilitating behavioural change is key. The role of the clinician in the herd scenario should be to facilitate understanding, to present potential solutions based on tackling the causal factor(s), and to help to prioritise actions.

In contrast, when using either the 'comparison with best practice' and 'differential diagnoses' herd diagnostic approaches, there is a limited ability to prioritise in the face of complexity. This prevents decision making based on the ranking of likely casual factors and the efficacy or efficiency of interventions. In addition, there is a danger that recommendations will be 'cherry-picked' by the farmer, leading to clinician frustration when key recommendations are not followed.

d. Summary

This epidemiological approach is used routinely by farm animal clinicians within University College Dublin. It is considered a generic and robust process. While it incorporates 'comparison with best practice' and 'differential diagnoses' investigative strategies, the initial focus on performance evaluation is closely aligned to 'the key abnormality of function' approach to individual animal cases. Whereas using the key abnormality of function approach is often regarded as unnecessarily complicated and time consuming in individual animal cases, the use of an epidemiological approach is easily justifiable during herd investigations as the ultimate outcomes are highly reliable in the face of complexities inherent in a herd-level focus.

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Genomic selection for animal welfare and environmental impact traits in dairy cattle

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Abstract

Genetic selection has led to phenomenal rates of genetic gain in milk production traits. However, from the mid-1990s, it was recognised that narrow breeding goals, focused on production traits, had negative consequences for fitness traits, impacting on animal welfare. The most obvious was the deterioration in female fertility, which has been observed worldwide. Since then, breeding goals have been extended and realised selection responses for traits such as fertility show that genetic selection can improve even low heritability traits. More recently, there has been a surge of interest in selecting for health traits and traits associated with resources, such as feed efficiency and methane emissions, even though the collection of these phenotypes may be expensive and only possible in small populations. In fact all these traits are ideal candidates for genomic selection where the basic requirement is a population that has genotypes and phenotypes (either on the individual or its progeny). Genotypes and associated phenotypes are used to create a reference population to calculate genomic prediction equations that can be applied to cows or bulls that are genotyped but do not have phenotypes. The opportunities to apply genomic selection to traits related to welfare and environmental impact are abundant, for example, feed efficiency, methane emissions and common health disorders, such as mastitis resistance, lameness and ketosis have large enough accuracies to be used in selection decisions. One option is to customise future breeding goals to include appropriate traits for the prevailing management and environmental conditions. For example, selection for heat tolerance is of great importance in emerging (and many existing) dairy regions. Genomic selection offers exciting prospects for genetically improving scarce and expensive to measure traits, such as those associated with dairy welfare and resource usage.

Traits under single gene control

In cattle breeding, there are many conditions that are determined by single genes, for example red coat colour is controlled by a recessive gene, so an animal has to have two copies of the recessive allele to be red in coat colour, if r denotes the recessive allele, rr genotypes will be red and Rr and RR will be black. Conditions such as polled follow a dominant mode of inheritance, in other words, individuals that are either heterozygous (one copy of the gene) and homozygous (two copies) are polled. There are also genetic diseases that have the same mode of inheritance, such as CVM, BLAD and DUMPS in Holsteins. Most of these disease are the result of reasonably recent (rare) mutations. For example complex vertebral malformation, or CVM can be traced to two former elite Holstein sires, because of their widespread use, the sires appeared on both sides of the pedigree of affected calves (Agerholm et al., 2001). Conditions such as these diseases highlight the importance of managing rates of inbreeding, which arises as a result of the co-occurrence of common ancestor(s) in maternal and paternal pedigrees.

Selecting for quantitative traits

Many of the traits that we deal with in animal breeding are polygenic in their genetic control. This means that the genetic architecture is often complex, involving many loci. Although it may be obvious that quantitative traits, such as height or weight are controlled by multiple loci, many diseases are also complex in their genetic architecture.

Genetic improvement programmes in cattle breeding have largely focused on quantitative traits. The models devised to do this analysis

have historically used pedigree information to devise an animal's genetic merit based on its ancestry and relationships with other animals in the dataset. Best linear unbiased prediction (BLUP) is the method that commonly does genetic analysis of cattle and has been the cornerstone of genetic evaluation programmes almost universally. Add to this the ease of exchange of genetic material worldwide in dairy cattle breeding, principally using AI, but also embryos and genetic improvement in many traits has been made.

Genomics

Genomic selection refers to selection decisions based on genomic breeding values (Meuwissen et al., 2001). The way it works is that a genomic reference population is assembled, typically genotyped bulls with large numbers of progeny. A genomic prediction equation is then calculated from the reference population by looking for associations between phenotypes and dense genetic markers that are approximately equally spaced across the entire genome. In cattle a variety of marker panels are used, the ones that are commercially used for genomic selection in have around 50,000 genetic markers. The genomic breeding values are calculated as the sum of each of the genotypes multiplied by its respective effect on a trait, thereby potentially capturing most of the genes that cause differences between animals in the traits of interest. This prediction equation can then be applied to individuals that are genotyped, but have no phenotypes. Therefore, the genetic merit of an individual can be calculated as early as birth and therefore selection decisions can be made earlier in life than traditional progeny-test approaches.

Genomic selection is now used routinely in many countries for genetic evaluation of traits that already have an estimated breeding value derived from a combination of pedigree and phenotype information (Spelman et al., 2013). The advantage of genomic selection for these traits is that the rate of genetic gain is accelerated by 40-50% (Spelman et al., 2103).

Selection objectives

Historically, the rationale to extend breeding goals to include new traits focussed entirely on their impact on farmer profitability. However, breeding goals are now becoming more complex in order to meet challenges set by consumers and society (Boichard and Brochard, 2012, Martin-Collado et al., 2015). Over the last couple of decades we have already seen a rapid evolution in the number of traits that are available for farmers to select on. Almost without exception these breeding values rely on large amounts of field data that are freely available through current recording systems, such as: milk production, calving records, insemination dates, pregnancy test outcomes, health records and culling dates. Common current breeding objectives in dairy cattle include: milk production traits, conformation, mastitis resistance through somatic cell count, longevity, calving ease, female fertility and workability traits.

The best way to ensure that all (measured) traits that contribute to profitability are included in selection decisions is to select the sires of the next generation using the local national selection index, examples include: EBI in Ireland, BPI in Australia, BW in New Zealand, PLI in the UK, NM in USA etc. An index is constructed by calculating appropriate weights for each of the breeding values included in the index. Historically the weights were calculated solely using economics, however more recently there has been interest in including farmers desires in the calculations e.g. (Martin-Collado et al., 2015).

Although selection for fertility, health, longevity etc has been practiced for over 30 years in Nordic countries (Heringstad and Østerås, 2013), for most countries, it was only from the 1990s that serious attention was given to selecting to improve other traits. Through the research happening at this time, it became clear that the observed deterioration in fertility and health traits was partly genetic therefore requiring a genetic solution.

Fertility

Fertility is one of the most important traits in dairy cattle breeding and the remarkable decline in fertility observed through the 1990s and part of the 2000s is partly attributable to narrow selection goals that focused on selection for milk production traits. Heritability estimates of traditional fertility traits are generally low (<0.1); yet selection for fertility can lead to worthwhile changes, because the trait is highly variable (Pryce and Veerkamp, 1999, Berry et al., 2014b). Between 40 and 64% of the deterioration in fertility is believed to be due to genetics VanRaden et al. (2004), (Berry et al., 2014b).

Pryce et al (2014b) used data collated by the World Holstein Federation to show that phenotypic calving interval appears to have reached a plateau by 2007. Between 1990 and 2000 calving interval increased "worldwide" by 1.25d/year phenotypically (Pryce et al., 2014b). The story of cow fertility has led to valuable lessons have been learnt by dairy geneticists in the dangers of narrow breeding goals. In addition to sustaining selection on fertility, welfare and disease resistance traits in particular are becoming key areas where breeding values are being developed for future breeding goals.

Health traits

In 1988, the first major review of data recording opportunities and consequently breeding strategies to improve production diseases was published (Emanuelson, 1988). However, since then computerised farm recording has led to a large increase in data available on these traits and consequently studies on genetic parameter and breeding value estimation.

Mastitis

One of the most important diseases in dairy production is mastitis. Selection to improve this trait is already being practiced in many countries through selection for reduced somatic cell count (SCC). Cell count can be quantified from routinely assessed milk samples and has a genetic correlation of around 0.7 with mastitis (Mrode and Swanson, 1996). However, several studies have shown that selection for mastitis directly is more effective than relying solely on predictor traits (such as SCC) (Heringstad et al., 2006, Egger-Danner et al., 2012, Gaddis et al., 2014). Currently the Nordic countries, Austria, Germany, Canada and France use clinical mastitis records in genetic evaluations (Egger-Danner et al., 2015).

In addition to SCC, other predictors of clinical mastitis could be used to increase the accuracy of breeding values. Examples include conformation (Lund et al., 1994), electrical conductivity from automated milking systems (Norberg, 2005) and lactate dehydrogenase which is a potential biomarker for mastitis (Friggens et al., 2007).

Lameness

Feet and leg issues are common reasons for culling in dairy cattle (Egger-Danner et al., 2015). Selection to reduce lameness has historically focused on conformation of feet of legs, as this is routinely recorded by breed societies and many countries already calculate breeding values for these traits (Table 1). However, the accuracy of breeding values for claw health or resistance to lameness increased when claw health data is included (Koenig et al., 2005).

Metabolic diseases

Metabolic disorders, such as ketosis, displaced abomasum, milk fever and tetany are disturbances to one or more of the metabolic processes in dairy cattle. Under-recording and difficulty in diagnosing subclinical cases are amongst the reasons why there is growing interest in using easily measurable predictors of metabolic diseases, either recorded 'on-farm' by using sensors and milk tests or 'off-farm' using data collected from routine milk recording (Pryce et al, 2016 in preparation).

Heat tolerance

Increases in ambient temperature, humidity, air flow and radiation exceeding the comfort zone are known to lead to heat stress in dairy cattle. This can lead to reduced appetite, production and compromised health and fertility. As with other traits, genetics offers a permanent and cumulative way to alleviate heat stress. Short-term heat stress an animal's appetite and therefore reduction in yield is often observed. It is possible to evaluate at what temperature-humidity load cows are affected and there is genetic variation in this trait. The heritability of heat tolerance around 0.11 (Nguyen et al., 2016; in press).

Feed efficiency and methane emissions

Feed is a major component of variable costs associated with dairy systems and therefore of great interest in dairy breeding objectives where the objective is to maximise profitability. However, it is expensive to accurately measure individual feed intakes on large numbers of cows. However, this makes feed efficiency an ideal candidate for genomic selection. As feed efficiency is so expensive to measure it is a good trait for international collaboration to establish an even larger reference population. In fact, (de Haas et al., 2012) combined dry matter intake phenotypes from Dutch and UK cows with Australian heifer phenotypes and found that the accuracy of genomic prediction was 5.5% higher when a multi-country reference population was used, compared to single-trait models. Since then, there has been further international collaboration through the global Dry Matter Initiative (gDMI) to build an even larger reference population (Berry et al., 2014a). The accuracy of genomic prediction is highest when a combined reference population is used (de Haas et al., 2015). Currently only Australia and the Netherlands have breeding values for feed efficiency that include feed intake data in their calculation.

Selection for reduced methane emissions is a growing area of interest. Recently, using a portable air sampler and analyser unit to measure methane emissions on 3,121 cows from 20 herds, Lassen and Løvendahl (2016) estimated that the heritability of methane emissions varied between 0.16 (s.e. 0.04) and 0.21 (s.e. 0.06). The genetic correlation with fat and protein corrected milk was high indicating that selection for production will lead to an improvement (reduction) in methane emissions. Selecting on traits that improve the efficiency of farm systems e.g. milk yield, residual feed intake and longevity will also have a favourable effect on overall emissions (Wall et al., 2010).

Conclusions

Selective breeding can be used to improve traits associated with welfare and climate change, ranging from traits controlled by single genes to those with complex genetic architecture.

Narrow breeding goals (i.e. selection for milk production only) leads to a deterioration of welfare related traits e.g. dairy cow fertility.

There is sufficient genetic variation in traits associated with animal welfare, such as health traits to make genetic progress in these traits feasible.

The most practical way to make genetic progress is to use the local national selection index e.g. EB1 in Ireland, BPI in Australia, BW in New Zealand, PLI in the UK, NM in USA etc.

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Bovine viral diarrhea viruses (BVDV) and their cousins the HoBi-like viruses:

Multi symptom, multi host, multi-tasking pathogens

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Introduction

The term bovine viral diarrhea (BVD) has come to refer to a diverse collection of clinical presentations that include respiratory, enteric and reproductive symptoms accompanied by immunosuppression. While the majority of cases are subclinical in nature two forms exist, mucosal disease and hemorrhagic syndrome, that are associated with clinically severe disease and high mortality¹. BVD may be caused by one of three different species of bovine pestivirus, bovine viral diarrhea virus 1 (BVDV1), BVDV2 and HoBi-like viruses²⁻⁵. Regardless of species, isolates may exist as one of two biotypes, cytopathic and noncytopathic. Isolates of the noncytopathic biotype, but not the cytopathic biotype, can establish persistent infections, which are the result of infection of the fetus during the first one third of gestation^{6,7}. While the primary hosts of these three viral species are cattle natural and experimental infections of a wide range of domestic and free ranging hosts are possible^{8,9}. All three species have been found as contaminants of fetal bovine sera. Control measures include detection and culling of persistently infected animals and vaccination^{10,11}. At present commercial vaccines and diagnostics are based on BVDV1 and BVDV2 strains and have limited efficacy for the detection and control of HoBi-like virus strains^{12,13}.

History and clinical presentation of BVD

The first reports of what subsequently was named BVD started with a call made by Dr. Francis Fox to a farm on the outskirts of Ithaca, New York in March of 1946 1996

¹⁴. Subsequent outbreaks on 5 additional farms, resulted in the recognition of "an apparently new transmissible disease of cattle"¹⁵. The presentations observed in these 5 farms reflect the mix of enteric, respiratory and reproductive disease that marks BVD outbreaks to this day. Immune depletion was observed regardless of clinical presentation. In the same year a disease outbreak was reported in Canada that had a number of similarities to the cases in New York¹⁶ (Childs, 1946), with exception that lesions were more severe, morbidity was lower and mortality was 100%. In retrospect the Canadian outbreak is recognized as the first published report of mucosal disease (MD)¹⁷. MD is a unique form of BDV that is the result of superinfection of an animal, persistently infected (PI) with a noncytopathic bovine pestivirus, with a cytopathic bovine pestivirus. While previous researchers had theorized that persistent infection was a lifelong consequence of fetal exposure, it wasn't until 1984 that persistent infections were reproduced under controlled conditions⁷. Those studies demonstrated that persistent infection could only be established if fetal exposure, in cattle, occurred prior to 125 days gestation and that persistent infection occurred following infection with noncytopathic viral isolates but not cytopathic viral isolates. It was also noted that PI calves had variable outcomes⁷. Some died as neonates while others survived the neonatal period but were stunted and suffered from poor thrift. Still others, while normal in appearance and surviving into adulthood, had a significantly higher rate of mortality due to respiratory or enteric disease compared to non-infected animals. It was noted that PI cattle succumbing to MD were infected with both cytopathic and noncytopathic BVDV¹⁸. Subsequently it was demonstrated experimentally that MD was the result of PI cattle being superinfected with a cytopathic BVDV^{18,19}.

Another form of BVDV appeared in North America in late 1980's and early 1990's²⁰⁻²³. The clinical presentation, which came to be known as hemorrhagic syndrome (HS) included severe pyrexia, bloody mucoid diarrhea and death within two weeks of initial clinical symptoms. It was found that the pestivirus isolates associated with HS belonged to a newly recognized species of pestivirus called BVDV2⁴. BVDV1 and BVDV2 strains differ both genetically and antigenically²⁴⁻²⁶. While several BVDV2 isolates were identified that caused HS^{4,27,28}, it was noted that infection with the majority of BVDV2 strains did not cause HS²⁹⁻³². In addition to a difference in the range of virulence following acute infections, the BVDV2 species has a slightly more restricted global distribution than the BVDV1 species, as BVDV2 isolates have not been detected in cattle in Australia.

Recently a new species of pestivirus associated with BVD, variously known as HoBi-like virus, bovine viral diarrhea virus 3 (BVDV-3) or atypical bovine pestivirus, has emerged. First described as a contaminant in a batch of fetal bovine serum imported into Europe from Brazil³³, these viruses were subsequently isolated from dairy cattle in Thailand³⁴, Brazilian water buffalos³⁵, aborted fetal bovine tissues samples from Brazil³⁶, calves suffering from respiratory disease in Italy² and cattle suffering from fever, diarrhea, and/or respiratory distress in Bangladesh³⁷. Surveillance studies in India detected more HoBi-like virus strains than BVDV1 and BVDV2 strains³⁸. Similar to BVDV1 and BVDV2 strains, noncytopathic HoBi-like virus strains can establish persistent infections^{5,39} and persistent infection can lead to mucosal disease⁴⁰.

The complete host range of HoBi-like virus is unknown, but under experimental conditions it can infect cattle, sheep, goats and pigs⁴¹. Because the earliest isolates of HoBi-like viruses were from samples collected from water buffalo and the two regions (South American and Asia), where HoBi-like viruses seem to have the highest prevalence, have significant populations of water buffalo it has been hypothesized that HoBi-like viruses in cattle result from a spill over from water buffalos. Of the three species of virus that cause BDV, HoBi-like viruses are the most geographically restricted with reported animal isolations in only three continents, Asia, Europe and South America.

Host range

Although most commonly associated with the infection of domestic livestock, based on natural and experimental infections, BVDV1, BVDV2 and HoBi-like viruses can replicate in a wide range of domesticated and free ranging ruminants (Table 1). The impact of bovine pestivirus replication in non-bovine hosts is not well documented. Bovine pestivirus infection of swine has significant economic impact in China^{42,43} and the detection of persistent infection in alpaca's in the US led to changes in health screening of alpaca for shows and breeding being instituted by the alpaca industry⁴⁴. The lack of regular surveillance programs, challenges in sampling wild populations, and scarcity of tests and vaccines specific for non bovine species compound the difficulties in detecting and controlling pestivirus infections in free ranging ruminants.

If infection of cultured cells is taken into consideration, the host range is even larger^{45,46}. In a survey of cell lines in the American Type Culture Collection (ATCC)⁴⁵ it was found that cell lines from cattle, sheep, goat, deer, bison, rabbit, and domestic cat were contaminated with BVDV. Attempts were made to experimentally infect 14 swine, rabbit, hamster, cat, dog, monkey, and human cell lines available from the ATCC that had been found free of virus in this survey. All swine cell lines, and most rabbit and some cat cell lines, became infected with BVDV while hamster, human, dog, and certain rabbit and cat cells were refractory to BVDV infection. It was assumed that the contaminated cell lines detected in the ATCC became infected due to the use of contaminated fetal bovine serum.

Table 1 Host range

Bovine pestivirus species	Primary host	Detected in free ranging wildlife species	Experimental in vivo Infections	Found as contamination in cell lines
BVDV1	Cattle	Bovidae, Cervidae, Leporidae, Antilocapridae, Camelidae	Bovidae, Cervidae, Leporidae, Antilocapridae, Camelidae, Suis	Bovidae, Cervidae, Leporidae, Suis, Felidae
BVDV2	Cattle	Bovidae, Cervidae, Antilocapridae, Camelidae	Bovidae, Cervidae, Leporidae, Antilocapridae, Camelidae, Suis	Bovidae, Cervidae, Leporidae, Suis, Felidae
HoBi-like	Cattle and Water buffalo	Not detected	Bovidae, Suis	Not detected

Contamination of reagents

The theorized introduction of HoBi-like viruses into Europe and the contamination of the ATCC via contaminated fetal bovine serum (FBS) highlight the problems resulting from bovine pestivirus contamination of reagents. A review of the literature between 1975 and 2012 revealed reports of the testing of 667 lots of commercial fetal bovine serum for infectious BVDV⁴⁷. The percentage that of lots that tested positive varied between 4% and 100% with an average of 29% containing infectious BVDV virus. Testing of 115 commercial lots for BVDV genomic RNA reported between 1996 and 2012 ranged from 46% to 100% positive with an average of 79% positive. This review of the literature noted that while BVDV is inactivated by gamma irradiation (30 kGy; equivalent to 3 Mrad), it was reported that the use of gamma irradiation failed to eliminate BVDV from a small percentage of FBS lots. Use of contaminated FBS in tissue culture propagation may give rise to BVDV infected cell lines and contamination of vaccines. As part of an international cooperative effort directed towards the improvement of the quality of medicines, the European Directorate for the Quality of Medicines of the Council of Europe (EDQM) organized a scientific symposium on pestivirus contamination of bovine sera in 2001. Based on the discussions at this meeting the (EDQM) organized a scientific symposium on pestivirus contamination of bovine sera in 2001⁴⁸. Based on the discussions at this meeting the committee for medicinal products for veterinary use (CVMP) revised their guideline on requirements and controls applied to bovine serum used in the production of immunological veterinary medicinal products to reads as follows;

4.3.3.2. Tests to detect Bovine Viral Diarrhoea virus

These tests should be carried out, a first time, before the inactivation treatment to assess the infectious titre of Bovine Viral Diarrhoea Virus potentially present to ensure it is below the level that has been shown to be effectively inactivated in the validation tests for inactivation treatment. Secondary tests should be performed after the inactivation treatment at which time no virus should be detected in the final serum batch (CPMP, 2003).

These guidelines are recommendations not regulations and it is not clear to what extent commercial FBS suppliers and biologics manufacturers follow them.

Similar to BVDV1 and BVDV2 viruses, HoBi-like viruses continue to be found as contaminants of commercial FBS to the present^{49,50}. Currently there are no recommendations or requirements testing for commercial FBS for HoBi-like viruses. Further, the sensitivity of HoBi-like viruses to gamma irradiation has not been established and standard testing methods are not recognized.

Conclusion

BVD eradication programs have been successfully enacted in some countries using control measures such as testing for and culling PI cattle and vaccination¹⁰. However, these programs do not address of the control of HoBi-like viruses in endemic regions or strategies to prevent the introduction of HoBi-like viruses into non-endemic regions. The diagnostic tests and vaccines used in current BVDV control programs were designed based on BVDV1 and BVDV2 strains and have limited efficacy against HoBi-like viruses. Further, current control programs focus only on domestic cattle and do not take into account the circulation of viruses that cause BVD in non bovine domestic species and free ranging species.

The proceedings from the International Symposium Bovine Viral Diarrhea Virus A 50 Year Review included this quote from one of the founding fathers of BVD research, Dr. Francis Fox of Cornell University;

“After observing and dealing with this disease for 50 plus years, I conclude that BVD is an interesting, intriguing, unpredictable, and most challenging disease which can quickly bring about financial disaster to the herd owner and apparently is going to be with us for some years to come.”(Fox, 1996)

It appears that Dr. Fox was not just a preeminent scientist but something of a seer as well. Just as he predicted, BVD is still with us and despite progress is still causing significant problems to this day.

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Managing Dairy Cow Health Through Nutrition

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Abstract

Nutrition's role in dairy cow health is, primarily, in the prevention of metabolic disease and in the management of chronic inflammation. Metabolic disease and associated premature deaths are a significant cost to dairy businesses, not to mention a considerable labour burden during already busy periods and, increasingly, an animal welfare concern. More than 90% of metabolic diseases occur during the transition between pregnancy and early lactation, and a mature cow is 6-times more likely to die during the month after calving than at any time during lactation. Diseases can be primarily divided into energy balance (EBAL)-related diseases, such as ketosis and fatty liver, or mineral-related diseases, such as milk fever, although most metabolic diseases are inter-related. Historical recommendations were to ensure cows were in positive EBAL before calving. More recent research results, however, indicate that if cows are a body condition score ≥ 3.0 (5-point scale), as is recommended, a positive EBAL before calving increases the risk of EBAL-related metabolic diseases and milk fever, and increases the concentration of inflammatory mediators circulating in blood. Instead, these cows should be managed to consume 80-100% of their daily metabolisable energy requirements during the two to four weeks before calving. In comparison, however, thinner cows (i.e., at a body condition score < 3.0) are at a low risk of EBAL-related metabolic diseases; if in negative EBAL before calving, these cows have higher concentrations of inflammatory mediators circulating in blood post-calving. Ideally, therefore, these cows should be managed to consume 100% of their metabolisable energy requirements before calving. Our increased understanding of the aetiology of these diseases will help aid in their prevention.

Introduction

Nutrition's role in dairy cow health is, primarily, in the prevention of metabolic disease and in the management of chronic inflammation. The majority of metabolic health disturbances occur between calving and three to four weeks (wk) post-calving. For example, Godden et al. (2003) reported that 14.5% of dairy cows culled in Minnesota exited the herd during the first 30 days (d) in milk, while Compton (personal communication) reported a three- to six-fold greater risk of death during the first month post-calving than at any other stage in the inter-calving period. It is generally accepted, therefore, that nutrition management during the weeks preceding calving is critical to reduce the risk of peripartum metabolic dysfunction, chronic inflammation, and exit from the herd during early lactation (Roche et al., 2013a) involving coordinated changes across multiple tissues and an enormous increase in nutrient requirements. Failure to transition successfully can result in reduced DM intake, milk production, delayed oestrus, failure to conceive and increased incidence of metabolic and infectious diseases, many of which are inter-related. Modern technologies have enabled the measurement of transcriptional changes in genes involved in multiple biochemical pathways across the transition period, enabling a better understanding of the implications of management and nutritional changes on cow health and productivity. Most recent research efforts have focussed on the association between pre-calving energy intake and postpartum health and productivity, with a general recognition that the positive relationship between pre-calving energy intake (and relevant circulating metabolites.

The six to eight wk transition between pregnancy and lactation (i.e., the transition period) has been extensively investigated and the major metabolic hallmarks characterising healthy and functionally compromised cows have been well described in numerous reviews (Goff and Horst, 1997; Drackley et al., 2001) ketosis, retained placenta, and

displacement of the abomasum-occur within the first 2 wk of lactation. The etiology of many of those metabolic diseases that are not clinically apparent during the first 2 wk of lactation, such as laminitis, can be traced back to insults that occurred during early lactation. In addition to metabolic disease, the overwhelming majority of infectious disease, in particular mastitis, becomes clinically apparent during the first 2 wk of lactation. Three basic physiological functions must be maintained during the periparturient period if disease is to be avoided: adaptation of the rumen to lactation diets that are high in energy density, maintenance of normocalcemia, and maintenance of a strong immune system. The incidence of both metabolic and infectious diseases is greatly increased whenever one or more of these physiological functions are impaired. This paper discusses the etiological role of each of these factors in the development of common diseases encountered during the periparturient period.”.

Many other reviews have summarised the diverse array of nutritional management strategies proposed to minimise metabolic dysfunction and the risk of disease in the periparturient period (Grummer, 1995; Horst et al., 1997)

Calcium transfer to the fetus in late pregnancy and the subsequent transfer of calcium to milk represent the greatest challenges to calcium homeostasis in adult animals. The adaptation of the maternal calcium homeostatic mechanisms is the result of a complex interplay between calcitropic hormones and the tissues, intestine, bone, and kidney, responsible for providing the large amounts of calcium needed to support fetal skeletal growth and lactation. In this review, we will discuss general calcium homeostasis followed by a review of the specific adaptations required by the human, rat, and cow to meet fetal and lactational demands for calcium. Finally, we will review what is known about the regulation of calcium transfer from the plasma to the milk.”.

(Grummer, 1995; Horst et al., 1997. Nevertheless, despite decades of trialling numerous nutritional strategies to maintain dry matter (DM) intake (DMI) and control the mobilisation of body stores of fat (i.e., body condition score; BCS), calcium, and, to a lesser extent, protein in early lactation, the incidence of metabolic disease and impaired performance remains high in both intensively managed confinement and more extensive pasture-based dairy production systems.

The purpose of this review is 1) to integrate recent research results with previous knowledge of transition cow metabolism and nutrition, and 2) to offer new insights into key elements of successful management of the transition cow, with particular emphasis on preventing energy balance (EBAL)-associated diseases, such as ketosis, and milk fever.

Historic advice – the need to feed: Steaming up transition cows!

Boutflour (1928) reported that, in his opinion, the “most serious limiting factors” influencing the cow's ‘yield of milk’ included “the neglect of the preparation of the cow for her lactation period”. In so doing, he defined the ‘Transition Period’ as a critical phase of the cow's lactation. He recommended that cows be fed an increasing amount of ration during the six wk before calving, with DMI of 4 to 6.5 kg DM, in addition to maintenance, required during the two wk before calving. Such a high pre-calving DMI became known colloquially as “steaming up” or “fitting” the cow, with the intention being to ensure cows were in positive EBAL before calving.

In support of the ‘steaming up’ concept, Hutton and Parker (1973) reported that cows that were fed to gain 0.7 kg live weight (lwt)/d during the four wk before calving produced 10-15% more milk during the first eight wk of lactation, when compared with cows fed to maintain lwt. More recently, Bertics et al. (1992) reported that cows force-fed through a rumen fistula before calving and into early lactation had lower concentrations of liver triacylglycerol (TAG) on the day of calving and produced 10% more fat-corrected milk during the first four wk of lactation when compared with cows that underwent a reduction in DMI before calving. Support for a

positive EBAL before calving also came from a positive relationship between pre-calving blood fatty acid (NEFA) concentration and post-calving disease incidence (Dyk, 1995). Therefore, it was concluded, that it is important to maintain or increase DMI before calving to prevent the net release of adipose tissue. These studies supported the need for a 'steaming up' ration before calving.

The peripartum profile of change in dry matter intake

In general, the "drive to eat" increases with energy requirements (Roche et al., 2008). A major exception to this rule appears to be the pre-partum dairy cow, whose DMI is reported to decline during the weeks preceding parturition (Bertics et al., 1992). 28 days before parturition, to examine the effect of ration energy level on the ad libitum consumption of energy in relation to requirements. During 25 days following parturition, 0, 2, 4, and 4 cases of left displacement of the abomasum occurred in the four ration groups, respectively. The mean daily dry matter intake 28 days prepartum did not differ significantly ($P < .05$) despite the increasing energy requirements for foetal growth and lactogenesis (Bell, 1995) decreased peripheral tissue glucose utilization, increased fatty acid mobilization from adipose tissue, and, possibly, increased amino acid mobilization from muscle. Within 4 d of parturition, mammary demands for glucose, amino acids, and fatty acids are severalfold those of the pregnant uterus before term. Even unusual postparturient increases in voluntary intake cannot satisfy this increased nutrient demand. Therefore, rates of hepatic gluconeogenesis and adipose fat mobilization are greatly accelerated. Concomitant changes in amino acid metabolism include increased hepatic protein synthesis and, possibly, decreased amino acid catabolism, and increased peripheral mobilization of amino acids. Insulin resistance in adipose tissue and muscle, developed during late pregnancy, continues postpartum; adipose lipolytic responsiveness and sensitivity to adrenergic agents are increased postpartum beyond their levels during late pregnancy. Before parturition, these homeostatic adjustments may be coordinated with lactogenesis by increased secretion of estradiol and prolactin. Their amplification and reinforcement at and soon after parturition may be regulated mostly by somatotropin.",

In recognition of this pre-calving decline in DMI and the putative need to have cows in positive EBAL, diets were formulated to increase energy density during the month before calving, primarily through reducing the forage to concentrate ratio.

However, the pre-calving decline in DMI is not universal. Roche (2006) and a failure to do so is reported to have negative implications on postcalving DMI (Grummer, 1995 reported no decline in DMI in the wks preceding calving in dairy cows consuming fresh pasture and hay, Coppock et al. (1972) 28 days before parturition, to examine the effect of ration energy level on the ad libitum consumption of energy in relation to requirements. During 25 days following parturition, 0, 2, 4, and 4 cases of left displacement of the abomasum occurred in the four ration groups, respectively. The mean daily dry matter intake 28 days prepartum did not differ significantly ($P < .05$) reported that DMI only declined pre-calving when concentrates exceeded 25% of diet DM, and Douglas et al. (2006) fat supplementation decreased dry matter (DM reported no decline in DMI pre-calving in cows restricted to 80% of energy requirements; however, in the latter study, DMI of cows offered unrestricted access to TMR declined during the three wks pre-calving. In fact, recent research from von Keyserlingk's group at University of British Columbia indicates that the pre-partum decline in DMI is associated with an impending malaise (e.g., metritis, ketosis) and not impending parturition, per se (Huzzey et al., 2007). If true in all cases, feeding activity could be a prognostic indicator of impending ill health. Taken together, these studies indicate that the reported decline in DMI before calving is not a 'natural' event associated with impending parturition.

The 21st century and a paradigm shift in thinking – are we killing the modern cow with kindness?

Despite the compelling evidence presented earlier to support the practice of 'steaming up' cows pre-calving, a closer examination of the results reveals inconsistencies. For example, in the study by Bertics et al. (1992), the high DMI cows in the force-fed group had 50% greater plasma NEFA circulating in blood and almost 100% greater plasma β -hydroxybutyrate (BHB) at 2 wk post-partum when compared with the low pre-calving DMI group. Considered in its entirety, therefore, Bertics et al. (1992) results indicate an increased risk of EBAL-related diseases with increased pre-calving DMI. These results do not support increasing DMI or diet energy density as the solution to the malaise indicated by elevated pre-partum NEFA (Dyk, 1995). Furthermore, studies designed to increase NEFA release before calving by restricting energy intake (Holtenius et al., 2003; Douglas et al., 2006; Dann et al., 2006) fat supplementation decreased dry matter (DM; Loor et al., 2006; Roche et al., 2005; Roche, 2007) 8.2, 10.0, or 11.0 kg/d of pasture dry matter per cow for 27 \pm 9.6 d precalving. This equated to 1.3, 1.9, 2.4, and 2.6% of body weight (BW; not including the conceptus weight provided no evidence of greater post-partum disease. In fact, cows that were feed-restricted for several weeks before calving had increased capacity for hepatic gluconeogenesis and β -oxidation, reduced hepatic TAG accumulation, and a reduced risk of milk fever and ketosis (Douglas et al., 2006; Loor et al., 2006; Roche et al., 2015) fat supplementation decreased dry matter (DM).

Recent research results indicate that cows overfed before calving have an inferior metabolic profile after calving (Douglas et al., 2006; Loor et al., 2006; Holtenius et al., 2003; Roche et al., 2005, 2015) fat supplementation decreased dry matter (DM. Overfed cows have higher NEFA and BHB concentrations, a lower post-calving DMI, and a greater negative EBAL during the two wk post-calving. This is a critical period, as 30-50% of BCS mobilisation occurs during this time (Roche et al., 2007) 352 daily BCS records across 3,209 lactations in 1,172 cows from a research herd in New Zealand. Mean daily BCS (scale 1 to 10 and factors that exacerbate NEFA release from adipose tissue are likely to increase the risk of metabolic disease. In addition, however, overfed cows have lower blood calcium on the day of calving (Roche et al., 2005, 2015; Roche, 2007) 8.2, 10.0, or 11.0 kg/d of pasture dry matter per cow for 27 \pm 9.6 d precalving. This equated to 1.3, 1.9, 2.4, and 2.6% of body weight (BW; not including the conceptus weight, implying an increased risk of milk fever.

To further complicate transition cow management, Roche et al. (2015) reported an interaction between pre-calving BCS and pre-calving DMI; the interaction implies that the benefit of the pre-calving feed restriction is through alleviating the risk of EBAL-related diseases associated with over-conditioning. The results indicated that:

- if cows with a BCS ≥ 3.0 (5-point scale) are restricted to between 80 and 100% of daily metabolisable energy requirements during the month before calving (i.e., a slight negative EBAL), they have superior profiles of metabolic and inflammatory-state (i.e., lower circulating concentrations of acute phase proteins and markers of inflammatory state) during the critical first two wk post-calving.
- cows with a BCS ≤ 2.5 , but in a similar negative EBAL to the high BCS cows before calving had an inferior inflammatory-state profile post-calving, even though plasma NEFA and BHB were lower.

This interaction is consistent with the large increase in the risk of EBAL-associated metabolic diseases with increasing calving BCS (Roche et al., 2013b). Garnsworthy and Topps (1982) were among the first to propose that historical recommendations for BCS were too high for modern dairy cows that were prone to excessive NEFA release in early lactation and, as a result, at a greater risk of EBAL-related diseases immediately post-calving (for review, see Garnsworthy, 2006). Combining a higher than optimal BCS (i.e., >3.0) with a positive EBAL before calving (i.e., steaming up) results in a failure to initiate the metabolic pathways

required for NEFA oxidation in the liver and increases the accumulation of liver TAG (Loor et al., 2006). In so doing, transition cows are at an increased risk of other metabolic diseases and premature mortality.

Summary – EBAL-related diseases

The aetiology of metabolic diseases is complex. However, two particular factors appear to interact to pre-dispose cows to a greater risk of EBAL-related disorders. The amount of NEFA released from adipose tissue increases and DMI declines post-calving with greater pre-calving BCS (Garnsworthy, 2006; Roche et al., 2009). This effect of BCS on the metabolic state of the transition cow is exacerbated by cows consuming energy in excess of requirements before calving. Releasing NEFA from adipose stores before calving primes the relevant tissues for NEFA oxidation, particularly the liver. As fatter cows (i.e., ≥ 3.0) release more NEFA during the first two wk post-calving, they are at an increased risk of TAG accumulation and associated liver dysfunction, which reduces the capacity of the liver for gluconeogenesis (Loor et al., 2006), increases the risk of ketosis, and exacerbates the peripartum chronic inflammation. Because of this, if cows are BCS ≥ 3.0 , they should be managed to consume approximately 80% of their requirements during the two to four wk pre-calving. If the diet is primarily long-chop forages (e.g., grass silage, hay, or fresh pasture), this restriction can be managed through reducing feed allowance (Roche et al., 2005, 2015; Janovick and Drackley, 2010) 8.2, 10.0, or 11.0 kg/d of pasture dry matter per cow for 27 \pm 9.6 d precalving. This equated to 1.3, 1.9, 2.4, and 2.6% of body weight (BW; not including the conceptus weight). However, if the diet is a finely chopped mixed ration, restricting DMI could increase the risk of displaced abomasum. Instead, a reduction in the energy density of the ration through the inclusion of straw or low digestibility hay or silage will facilitate the reduction in energy intake. Cows that are thinner than BCS 3.0 are at a low risk of EBAL-associated metabolic disorders and should be fed to requirements.

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The Role of Veterinarians in Udder Health Programs

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Introduction

Throughout the world, the structure of dairy farms is evolving. In 2015, milk production in the E.U. increased by about 5%, with Ireland experiencing the greatest increase in wholesale milk deliveries. In the U.S., consolidation of dairy farms has occurred at a rapid pace. In 2012, dairy farms containing more than 500 cows comprised only 6% of all U.S. herds, yet produced 63% of total U.S. milk output. As the dairy industry has globalized, the trend toward larger dairy herds is accelerating and has influenced the role of veterinarians. On larger dairy farms, cows are managed in groups, workers are specialized and farm owners often have apparently free access to a variety of technical services provided by agribusinesses. These changes in farm structure are influencing demand for veterinary services. Historically, most dairy veterinarians provided a "herd health" program that focused on managing fertility (Derks et al., 2012) and pregnancy examination has been the primary reason for routine herd visits. However, many aspects of reproductive herd health programs are now being performed by paraprofessionals (Mee, 2007b) and the loss of a reason for veterinarians to routinely visit farms can result in decreased opportunities for consultation on other topics. The objective of this paper is to review the current role of veterinarians in udder health programs and propose a framework for enhancing that role to meet the evolving needs of dairy farmers and consumers.

Perceptions and Reality of Veterinary Engagement in Udder Health Programs

The purpose of dairy herd health programs is to prevent animal disease, improve animal well-being, reduce treatments and help ensure profitability of the dairy business. Most research about herd health programs consistently indicates that relatively few veterinarians offer comprehensive udder health programs. Only 24% of dairy farmers who enrolled in a milk quality program in Wisconsin, indicated that they routinely used their veterinarian to plan milk quality programs (Rodrigues et al., 2005a) and 3-4 years after completing the, only 38% of the farmers continued working with veterinarians on udder health (Hohmann and Ruegg, 2012). In a separate survey, most dairy veterinarians indicated that they spent <10% of their professional time actively working to improve milk quality (Rodrigues and Ruegg, 2004). In England, the most common response of veterinarians was that they did not provide regular veterinary advice about mastitis control to any farms but many indicated that they occasionally provided some monitoring. In The Netherlands, udder health was listed as the 3rd priority for herd health and about 50% of dairy farmers believed that veterinarians were not aware of or were ignoring their goals for herd health (Derks et al., 2012).

In contrast to reproductive health programs, implementation of an udder health program often requires input from other farm advisors and application of problem solving rather than clinical skills. Veterinarians frequently overemphasize possessing technical knowledge over marketing and communication skills (Kristensen and Enevoldsen, 2008). Barriers to improvement of milk quality are primarily related to motivation and implementation rather than lack of technical knowledge or skills (Rodrigues and Ruegg, 2005, Jansen et al., 2010, van den Borne et al., 2014). One reason may be that veterinarians typically have similar personality types that tend to overemphasize logical, organized and practical approaches to the world (Johnson et al., 2009). These traits are important for clinical decision making, but are not skills that are generally associated with individuals who are effective at convincing farmers to invest in udder health programs.

Numerous surveys have demonstrated that veterinarians and dairy farmers often have very different perceptions of each other's goals

(Kristensen and Enevoldsen, 2008, Lievaart et al., 2008, Lam et al., 2011, Hall and Wapenaar, 2012, Derks et al., 2013). In comparing responses of farmers and veterinarians about components of a herd health program, virtually all responders agreed that reproductive health monitoring is part of a herd health program, while fewer farmers included routine monitoring of udder health (Lievaart et al., 2008). Many farmers are price sensitive and value efficient use of time, thus programs that are perceived to be time consuming and provide low financial returns are often discontinued (Derks et al., 2013). Without appropriate presentation and marketing of udder health programs, only the most motivated farmers will routinely participate.

Opportunities for Veterinarians to Engage in Udder Health Program

Reasons that veterinarians fail to engage in reproductive herd health programs include lack of demand, failure to justify cost lack of confidence and competence, lack of desire to proactively engage with other service professionals and lack of financial incentives (Mee, 2007a). These same reasons likely apply to veterinary engagement in udder health programs, however there are plenty of reasons for veterinarians to increase involvement. It is well known that mastitis has a profound impact on farm productivity and animal welfare. The occurrence of mastitis reduces milk production, increases the amount of milk discarded and premature culling and costs (Fetrow, 2000). Veterinary involvement in udder health programs is often limited to reacting to increased bulk tank SCC, but there are tremendous opportunities for veterinarians to provide more services. Antibiotic use in livestock is an increasing concern and control of mastitis accounts for the majority of antibiotics used on dairy farms, (Pol and Ruegg, 2007, USDA, 2008, Saini et al., 2012, Gonzalez Pereyra et al., 2015). Increased involvement of veterinarians in oversight of treatment protocols for mastitis is a logical starting point and this opportunity can serve as the platform for beginning more comprehensive involvement in consultative services.

While some countries require veterinary supervision of antibiotic treatments (Lind et al., 2012), in many regions, administration of treatments for mastitis are the responsibility of farm personnel and veterinarians are involved only when a case becomes life-threatening (Richert et al., 2013). While prescribing drugs is usually a veterinary responsibility monitoring compliance and efficacy is often neglected. Ideally, the veterinarian collaborates with farm workers to develop and evaluate farm specific treatment protocols. The veterinarian also has a role in ensuring that treatment protocols are effectively delivered. On large farms, the veterinarian can be an important resource for training farmers to collect milk samples and to properly administer treatments. The veterinarian can also provide oversight of on-farm laboratories used to guide treatment decisions. While other service providers often have specialized knowledge about nutrition, breeding and milking, only veterinarians have specific training in immunology, mammary physiology, pharmacology and microbiology. These unique skills should be leveraged by veterinarians to provide services that facilitate implementation of an annual udder health plan.

Components of an Annual Udder Health Plan

Veterinarians have long been encouraged to deliver comprehensive herd health programs that include monitoring performance, prescribing corrective actions and evaluation of outcomes. The challenge is finding time to provide and be compensated for these services. When aspects of an udder health program are considered, there is a long list of areas of potential veterinary involvement (Table 1). However, veterinarians are not considered the first source of expertise for some components and may compete with other service providers who do not appear to directly charge for their services. Among all potential components of an udder health program, use of effective treatment protocols that ensure justifiable use of antibiotics is the area that is most unique to veterinary expertise and veterinarians need to increase involvement

Table 1. Potential components of an annual udder health monitoring plan (adapted from Ruegg, 2011).

Potential areas of involvement for veterinarians	Components of Annual Udder Health Plan	Examples of compliance indicators or key performance indicators ¹
Ensuring justifiable use of antibiotics	Identification and training of farm workers authorized to administer antibiotics	Signed training documents for authorized farm workers
	Development, review and revision of all treatment protocols	Review of all authorized treatment protocols based on assigned expiration dates
	Monitoring compliance with treatment protocols	Number of days of treatment and milk withhold
	Regular review of efficacy of antibiotics used in mastitis treatment protocols	Recurrence of CM, SCC reduction, proportion of cases requiring treatment changes, etc.
	Drug residue avoidance	Monthly review of withholding days for milk and meat of cows treated in month
Monitoring clinical mastitis	Incidence rate	<25 new cases per 100 cows per year
	Recurrence within 60-90 days	<20% of incident cases
	Distribution of severity scores,	50% mild, 35% moderate, <15% severe
	Case fatality rate	<2% of clinical cases
	Identification of chronicity	<5% of cows milking on <4 ¼
Monitoring subclinical mastitis	Bulk Tank SCC	<250,000 cells/mL
	Prevalence (% > 200,000 cells/mL)	<15% of cows
	Incidence (cows with SCC>200,000 cells/mL for 1 st time in lactation ^a or change from previous month ^b)	<5 ^a -8 ^b % new SCC>200,000 cells/mL ²
	Prevalence in early lactation (measured at 5-40 days in milk)	<5% of 1 st parity cows with SCC>200,000 cells/mL
	Prevalence in late lactation (measured in last month before dry off)	<30% of cows with SCC>200,000 cells/mL
Bacteriological quality of bulk milk	Total bacterial count (TBC), Laboratory pasteurized count (LPC), Preliminary incubated count (PIC), Coliform count	<10,000 cfu/mL TBC; <200 cfu/mL LPC; <10,000 cfu/mL (PIC); <100 cfu/mL (coliform count)
Use of appropriate diagnostic methodologies	Bulk tank culture	0 cfu of Strep ag, Staph aureus & Mycoplasma bovis
	Culture guided selective treatment for clinical mastitis (on-farm or in-veterinary clinic)	see guide at milkquality.wisc.edu
	Diagnosis for selective dry-cow therapy programs	Targeted culture-based programs
	Appropriate use of molecular technologies	Guided use of PCR and other technologies
Preventive programs	Monitoring hygiene	<20% udder hygiene scores "dirty"
	Monitoring milking protocols	Prep-lag time of 60-120 seconds; >90% of teats adequately covered with post-dip
	Monitoring bedding/pasture quality	Review bacterial counts of bedding
	Management of dry cows & heifers	SCC in early lactation (see above)

¹Suggested KPI for some indicators are available online: <http://milkquality.wisc.edu/> ; ²depending on method of calculation

in this area. The development of an annual udder health plan that is initially structured to focus on this aspect is one mechanism for local veterinarians to engage producers in managing udder health.

A Framework for Prioritizing Veterinary Engagement in Udder Health Programs

The focus of udder health programs should be on prevention of new infections and a comprehensive udder health program will require inputs from all relevant advisors and is best implemented in a team-based setting. Farmers consistently indicate that they value teamwork and goal setting (Kristensen and Enevoldsen, 2008, Lind et al., 2012) and participation in team-based herd management programs have been shown to result in benefits to dairy farmers (Rodrigues and Ruegg, 2005). Leadership of an annual team-based udder health plan is an obvious mechanism for veterinarians to leverage the strengths of various consultants while maintaining focus on ensuring justifiable use of antibiotics. Annual udder health plans should be formulated in a team-based setting and include reasonable goals that are arrived at by consensus of team-members, a limited number of actions and methods to evaluate the results of the actions, assignment of responsibilities to team members to accomplish actions and target dates to meet and evaluate progress. Simple forms (on paper, tablets or laptops) are often useful for guiding the programs (forms are available online: <http://milkquality.wisc.edu/programs/milk-money/>). Experience with a team based milk quality program has shown that this simple process can be successful in encouraging adoption of best management practices and increasing involvement of veterinarians in udder health programs (Rodrigues et al., 2005a, Rodrigues et al., 2005b). The process is simple and can initially require as little as 4 hours of management time per year. A team of advisors agrees to meet to formulate the plan, and schedules a series of 1 hour meetings that are focused strictly on udder health (depending on herd goals, the interval between meetings may range from 1-4 months). At the first meeting the team sets initial goals for udder health and assigns actions to various team members to complete. At least one goal should be permanently reserved for development, implementation and evaluation of treatment protocols that limit unnecessary use of antibiotics. On many farms, the first step in development of a treatment plan is to ensure that an appropriate mastitis recording system. A review of antibiotics usage should be performed and practitioners should periodically recheck which antimicrobials are being used and if farmers are in compliance with governmental regulations. At subsequent meetings, the action list is reviewed and people are held accountable for completion of their tasks. Farmers who participated in this type of program indicated that they increased consultation with their veterinarian about milk quality from 20% (before program) to 84% (at the end of the 4 meetings) (Rodrigues and Ruegg, 2005). Analyses of herds that completed a team based goal oriented program indicated that this method can result in rapid improvement in milk quality (Rodriguez and Ruegg, 2005).

Conclusion

Veterinarians have an opportunity to increase involvement in udder health programs and the development of an annual udder health plan is a mechanism that can be used to overcome barriers to veterinary engagement. There are a number of key influencers who often work with dairy farmers in various aspects of udder health and rather than compete to provide services, the formation of a team-based udder health plan can leverage knowledge, skills and resources to better serve the farmers needs. The development of a team-based annual udder health plan also provides a mechanism for veterinarians to evaluate treatment protocols to ensure that all antibiotic usage is justifiable and appropriate.

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Small ruminants: opportunities for improved production efficiency to sustainably meet the needs of the world's human population for food.

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The role of small ruminant farming

Having evolved as selective browsers in resource poor, semi-arid or humid ecological environments, goats are generally more robust and efficient than other domestic ruminant species in their metabolism and tolerance of poor quality and potentially toxic nutrients and conversion into food products. Sheep have evolved to be particularly well adapted to convert short herbage to milk or meat. Different small ruminant breeds and production systems have been developed to suit local resources in seasonally biodiverse environments throughout the world. Small ruminant production is, therefore, suited to both advanced agricultural systems on productive, fertile land, and to environments that are harsh, or seasonally deficient in energy, nitrogen, phosphates, water and organic manure. Small ruminants are consequently adaptable to meet global needs for food security and have potentially important roles in improving the health and wellbeing of the rural poor in their marginal environments.

Small ruminants are generally better suited to enhancing the livelihoods of the poor than cattle and buffalo. This is due to their manageable size, relatively low maintenance requirements per unit of product, low capital investment cost, short generation interval and ease of marketing of animals and products, hence suitability as short-term economic reserves. Small ruminant farming is widely portrayed to be a solution to the challenge of achieving socioeconomically and environmentally sustainable global food security in the face of effects of population growth, urbanisation and affluence, vulnerability to climate change and the hitherto irresponsible agricultural use of drugs and chemicals.

Efficiency of small ruminant farming

Global small ruminant farming is generally inefficient and unsustainable with reference to utilisation of the world's finite resources to provide food for its burgeoning human population. There is a fundamental requirement to meet the daily nutritional requirements of farmed livestock for maintenance, before any nutrients can be converted into food for human consumption. Consequently, inefficiently produced animals must digest and metabolise more nutrients than efficiently managed counterparts to produce the same yield of meat or milk product. Inefficient production is generally uneconomic and not conducive to achievement of good states of animal welfare, hence unsustainable and socially unacceptable. Furthermore, inefficient farming causes environmentally unsustainable levels of greenhouse gas production, arising from the level of forestomach microbial digestion per unit of food produced.

The economic potential of commercial small ruminant farming in developed agricultural systems and the role of small ruminants in alleviating poverty in seasonally resource-limited regions are frequently not realised. This situation pertains despite advances in animal breeding and genetics, sustainable husbandry and animal health management. Failure to translate research and development into efficient utilisation of natural resources in target environments must be investigated and addressed before small ruminant farming can be exploited as a solution to the challenge of socioeconomically- and environmentally-sustainable global food security.

Ecological impact of small ruminants

Small ruminant farming has contentious ecological effects, such as alteration of natural flora caused by their characteristic selective short grazing or browsing behaviour. In scenarios such as those where small ruminants subsist on unimproved natural herbage with low levels of management intervention, this has the potential to result in environmental degradation. Conversely, in some managed goat herds and sheep flocks, this selective feeding behaviour affords opportunities to create suitable environments for other animal species, for example enabling synergistic co-grazing with cattle or creating environments suited to wildlife conservation management. Co-grazing small ruminants with other domestic herbivores in this manner can have both positive and negative animal health benefits. Selective clearance of vegetation by goats may help to limit the habitats of arthropod disease vectors; while goats may act as reservoirs of infection for more susceptible co-grazed hosts, or vice versa. Furthermore, in some agricultural production systems, small ruminants may be an ecologically-friendly source of manure. The net ecological impact of small ruminant farming on other forms of agricultural or biological production, wildlife and the natural environment are poorly understood. Better knowledge of consequences of interactions within whole farming systems is required to define and address sustainability in these regards.

Genetic improvement and technological advance

To-date, the main focus of attention to redress failures of small ruminant production to meet global needs for food security has been upon genetic improvement, through breed replacement, breed crossing heterosis and, or, within breed selection. This has been in conjunction with the development of new technologies, such as automated electronic recording systems, or reproductive management tools. In some regions small ruminant breeding schemes have been established, or proposed, to provide farmers with genetically improved animals capable of larger litter sizes and, or, faster kid growth. Arguments have been made to identify and genetically capture beneficial *de novo* mutations, thereby editing genes to engineer animals that are tolerant of diseases for which pharmaceutical or vaccine control is ineffective or unsustainable. These technologies will inevitably become essential, as over the next 50 years, the United Nations Sustainable development Forum predicts that the world's farmers will need to produce more food than in the past 10,000 years combined. However, genetic and genomic progress will only succeed if the animals are first kept alive and productive by applying scientific health planning principles to integrate animal genetic selection with good nutrition, husbandry and disease management.

Genetic improvement has had a major impact on pig and broiler poultry industries. Similar levels of improvement in small ruminant genetics are theoretically possible allowing small ruminant farming to become sustainably efficient within 10 years. However, the based on the slow rate of improvement over the past 10 years, the likelihood of achieving this potential is low. Genetic improvement in pigs and broiler poultry has been successful because it has been matched by appropriate nutrition and animal health management. However, precise nutritional and animal health management is easier in closed groups of housed pigs and poultry fed on cereal based rations, than it is in pastorally managed small ruminants.

The solutions to some of the nutritional and animal health constraints to achieving the potential of genetic improvement are known. Hence there is an opportunity for improvement in global food security through the development of impactful educational tools for small ruminant farmers or keepers and their veterinary advisors. However, the practical application of solutions to many nutritional management and animal health constraints are unknown, requiring applied research to allow the potential of genetic improvement to be realised.

Planned small ruminant health management

Unsatisfactory commonplace scenarios, such as high rates of neonatal mortality, poor growth rates and poor reproductive performance in small ruminants can only be addressed by applying scientific health planning principles to identify and manage primary causes, rather than the common practice of deferring to pattern recognition. Veterinary medicine must change to allow the scientific application of health planning approaches to address suboptimal production in groups of animals within the overall context of their environment, husbandry, management and contemporary agriculture. A fresh perspective of targets, management and disease control is needed, with a focus on the responsible and sustainable use of remedies, rather than a dependence on pharmaceutical treatments.

The iterative principles of planned animal health involve: i) benchmarking the productivity of the animals against tangible targets; ii) identifying any constraints and rationally investigating their causes; iii) ensuring that the most appropriate remedies are used efficiently, based on assessment of the individual circumstances; iv) evaluating the response to and cost benefits of whatever management or remedies are employed; and v) continuing to monitor and address issues repeating the process as indicated to ensure ongoing improvements in productivity and welfare. As the process advances, the application of state-of-the-art scientific research becomes increasingly important in ensuring optimal efficient and sustainable productivity.

Having determined that key small ruminant production indices do not meet pragmatic system-specific targets, the next step is to identify the primary constraints. This involves: i) evaluation of the problem history; ii) assessment of environment; iii) examination of animals; and iv) the use of practical diagnostic tests and expertise in the interpretation of their results. However, the global availability of appropriate and validated tests for the diagnosis of many important production limiting diseases is limited. This can be a barrier to useful disease diagnosis, regional and national disease surveillance, the development of disease management strategies, and national and international disease control programmes. Accurate diagnosis is important to allow the application of state-of-the-art scientific research, for example concerning genetic selection for production and resistance to disease constraints, or use of genomic markers to monitor and develop treatment, management or eradication strategies.

Having first identified primary animal health constraints, the practicality, appropriateness and cost benefits of remedies must be evaluated. It is necessary to determine the balance between the cost of forage production, or labour inputs required to tend animals on free-access herbage, and the nutritional value, while also considering the impact of the animals on the overall ecosystem to which they belong. The response and cost benefits of infectious disease control need to be evaluated.

Small ruminant nutritional management

The primary reason for keeping small ruminants is to convert primary herbage or other crop resources into marketable meat, milk, or fibre products. Hence, inadequate nutritional management has direct effects on economic production efficiency. Inadequate nutritional management has consistently been shown to be a primary obstacle to achieving the genetic potential for production of small ruminants.

The nutritive values of a range of natural herbage and of cultivated crops, are well known and the principles of ration formulation are understood. However, as a general principle, the nutritive value of the ration that is actually fed or ingested is often misjudged due to seasonal variation in the availability of nutrients, poor understanding of the energy cost associated with growing or feeding herbage, or changes in availability as a consequence of urbanisation and other demands on grazing and crop producing land. There is a tendency to compensate for seasonal variations in availability of natural and cultivated herbage through feeding of cereals. However, concentrate feeding induces a reduction

in the pH of the forestomach contents, potentially reducing the efficiency of digestion of primary herbage resources. Similar considerations apply to the growing practice of energy crop by-product feeding. There is therefore a need for better understanding of the nutritive value of herbage that is fed to small ruminants, of the concept of a balanced ration, and of the effects of concentrate feeding on the efficiency of digestion of primary natural nutrient resources.

Undernutrition can weaken host protective immune responses to infectious diseases such as helminth, arthropod and protozoal parasitism, or contagious viral diseases, with indirect consequences on economic small ruminant production efficiency. Conversely, the consequences of poor nutrition are commonly compounded by inadequately managed disease constraints. It is therefore important to recognise the impact of different constraints to production efficiency on each other. For example, diverse problems of high nematode parasite burdens and poor reproductive performance may be consequences of the animals being in poor body condition and negative nutritional balance. Consequently, improving the efficiency of utilisation of seasonally available herbage resources may be a practical approach to addressing parasitological or reproductive problems. This might be achieved by introducing a compact, seasonal lambing or kidding pattern, following the model that is prerequisite for pastoral cattle production. A compact reproductive period further enables targeted disease management, for example helminth control, and targeted grazing and nutritional supplementation management, in line with the global move towards precision livestock farming systems. However, before promoting such a fundamental change in animal husbandry, it is first necessary to understand the seasonal nutrient availability and deficiencies in conjunction with compounding effects of infectious disease threats.

Infectious disease management

Infectious diseases such as lameness, mastitis, respiratory disease, or slow onset causes of ill thrift in adult animals are important constraints to efficient small ruminant farming in many systems. These diseases are generally predisposed to by specific management practices. Hence, addressing the predisposing causes is generally more effective for their control than reliance on antimicrobial drugs. The threat of antimicrobial drug resistance raises concerns about dependence on whole group prophylactic or metaphylactic treatments.

Infectious disease plagues such as foot and mouth, bluetongue and peste des petits ruminants (PPR) are a serious problem in many parts of the world. The solutions to these problems lie in the development and application of rapid diagnostic tests, effective vaccination regimes, or eradication.

Global importance of parasites to small ruminant farming

Eleven of the World Health Organisation's seventeen Neglected Tropical Diseases (NTDs) of humans are parasitic infections (Chagas disease; Dracunculiasis; Echinococcosis; Food borne trematodiasis; Human African trypanosomiasis; Leishmaniasis; Lymphatic filariasis; Onchocerciasis; Schistosomiasis; Soil transmitted helminthiasis; and Taeniasis/cysticercosis). A further NTD is thought to be mechanically spread by parasitic vectors (Tachoma). There is limited evidence to show the net impact of parasitic diseases on small ruminant production. However, parasitic diseases are widely considered globally to be as important in small ruminants as they are in humans, being the foremost animal health constraints to efficient production. Eight of the NTDs are also classified as Neglected Zoonotic Diseases (Echinococcosis; Taeniasis/cysticercosis; Foodborne trematodiasis; Human African trypanosomiasis; Leishmaniasis; and schistosomiasis), demonstrating a direct human health priority to control parasitic diseases in livestock.

Globally diverse climatic conditions provide various opportunities for the survival and development of free living and environmental stages

of a range of arthropod, protozoa and helminth parasites; while large livestock populations and diverse agricultural management enable completion of the parasite life cycles. Consequently, tick, mite, fly, louse and flea infestations all occur commonly in small ruminants. Vector borne diseases, including haemoprotozoa and filarial nematode infections are particularly problematic where warm and humid environmental conditions favour temporary parasites. Protozoal parasite infections such as cryptosporidiosis, coccidiosis and toxoplasmosis are commonplace because intensive and sometimes unhygienic management favour host infection; while seasonally warm and wet climatic conditions, or drinking water supply management are conducive towards oocyst survival. Nematode infections are commonplace where environmental conditions and livestock management result in the development of high infectious larval challenge; and haemonchosis is a major production limiting disease in regions with seasonally warm and wet climates. Trematode parasites such as *Fasciola* spp. and *Dicrocoelium* spp. liver flukes, amphistome rumen flukes and *Schistosoma* spp. blood flukes cause disease in animals kept in environments that favour parasite infection and development in the intermediate hosts. Small ruminants are both intermediate, and definitive hosts to a wide range of cestode parasites including *Taenia* spp and *Echinococcus* spp., and *Monezia* spp., respectively. The principles of sustainable parasite control in the face of changing epidemiology brought about by socioeconomic and climatic changes are, therefore, important with reference to ensuring global food security.

Nematode parasites

Nematode parasites cause significant economic losses to farmers and their keepers in terms of reduced production and treatment costs, as well as being a major welfare issue for the infected animals. *Haemonchus contortus* is arguably the greatest cause of poor production efficiency in small ruminants globally, causing ill thrift, anaemia and death. Gastrointestinal trichostrongyle nematodes such as *H. contortus* have direct life cycles, involving non-parasitic environmental egg, first, second and third stage larvae, and parasitic third and fourth stage larvae, and dioecious, sexually reproducing adults. The biology of these parasites is, therefore, influenced by both environmental and host factors, which must be understood and accounted for when planning for their control.

The aim of all approaches to nematode parasite control is to limit the host challenge to a level, which does not compromise performance or welfare, while at the same time enabling the development of protective immunity. Sustainable nematode control in individual flocks or herds must, therefore, be underpinned by knowledge of the farming system and of the relationship between pasture contamination, availability of infective larvae and the build up of infection in host animals. There has been a tendency to focus on prescriptive approaches to the control of nematode parasites, which are not always founded upon knowledge and understanding of these principles. In fact, given the economic impact of nematode parasites and complexity of the principles underpinning their control, most farmers and small ruminant keepers, worldwide, rely upon the use of anthelmintic drugs in an unsustainable attempt to treat infected animals. Such treatment frequently involves routine prescriptive anthelmintic treatments of individual animals, or mass drug treatment camps.

Current prescriptive approaches to the control of nematode parasites are generally ineffective and inevitably unsustainable. *H. contortus* resistance to benzimidazole, imidazothiazole, macrocyclic lactone drugs is now commonplace, highlighting the need for iterative flock and herd health planning, incorporating evasive nematode control and strategies aimed at slowing the emergence of anthelmintic resistance. The principles of sustainable nematode control in the face of changing epidemiology brought about by socioeconomic and climatic changes are poorly understood. Advances are needed in understanding these principles in small ruminant production systems to address the global need for efficient livestock production. The publication of a high quality

draft *H. contortus* genome and transcriptome affords opportunities to combine veterinary clinical investigation of nematode parasitic diseases with post genomic research to identify impactful solutions to the challenge of sustainable nematode control.

Education

There are many unknown factors with regards to sustainable nutritional management and parasite control, hence a need for applied research. However, in many cases poor production efficiency is caused by simple animal husbandry problems such as poor basic hygiene and colostrum management. The solutions to these challenges are already known, as they are for aspects of nutritional management and parasite control. Hence there is a requirement for the development of tools and systems, such as the 'train the trainer' concept, aimed at translation of knowledge of good animal husbandry, health planning, principles of disease control and mitigation of zoonoses for small ruminant farmers and keepers.

Summary

There are many reasons for the unsustainability of global small ruminant production to meet the requirements of small ruminant farmers and keepers for both subsistence and profit. The principal opportunities for improvement are afforded by integrating animal genetic selection with good nutrition and health management. Genetic selection criteria have tended towards particular traits such as milk production, carcase quality or growth rates, but have often failed to consider the suitability of individual animals to their target environment, with reference to nutritional and disease constraints. Improved animal health not only addresses the issue of food security, but also has obvious socio-economic and environmental benefits and improves human health standards through the control of zoonotic diseases. An integrated approach founded on robust scientific principles is required to apply precision farming methods to combine genetic potential with appropriate feeding strategies and planned animal health management.

Benchmarking Animal Welfare: Engaging producers and improving practice²

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Introduction

Our research group has worked for some 20 years to identify methods of improving the welfare of dairy cows. Projects vary depending upon farmer and student interest, funding, etc., but one common feature is that we attempt to address real world problems faced on dairy farms, and provide practical solutions that could be adopted by at least some farms. The unhappy truth, however, is that the gap between study and changed practices on commercial farms can be immense. This failure to get research into practice can be due to a number of factors. Maybe the research question was not really relevant to farmers? Or maybe the researcher has just not worked hard enough to tell farmers about their work and how it might be useful?

We have become interested in developing new ways of closing the gap between science and changed practice on farms. The aim of this paper is to describe one such approach. Below we will argue that 'benchmarking' welfare relevant measures is effective in getting farmers interested in problems, and that this method can lead to changed practices on farms resulting in better lives for the animals. We suggest that benchmarking works because it provides the farmer with real data from their own farm and places this data into context by comparing the results with those of other farms in the same region. We have also found that this approach allows for new conversations between the farmer and the professional team they work with, including their veterinarian. In this way benchmarking may provide veterinarians a new way to get their clients interested in specific issues, and to help their clients develop tailored solutions to the problems.

Lameness is perhaps one of the greatest threats to dairy cow welfare; lameness is painful, long lasting and prevalent. Our group's work on lameness has been closely linked to our research on cow comfort; we believe that by building better barns for dairy cows, barns that are more comfortable for the cows to use, we can reduce injuries on the legs and hooves of the cows, and ultimately reduce the rates of lameness. In many studies using our own research farm we have tested various design and management features to see how these affect cow comfort (e.g. Abade et al., 2015; Winkler et al., 2015), injuries (e.g. Proudfoot et al., 2010; Dipple et al., 2011) and lameness (Hernandez-Mendo et al., 2007; Bernardi et al., 2009). For example, a series of studies have shown that cows strongly prefer lying down in stalls with deep, dry, and well maintained bedding (Tucker et al., 2009; Fregonesi et al., 2007; Drissler et al., 2005). We have also found that cows are motivated to find a comfortable place to stand (ideally a soft, dry standing surface), and that providing a more comfortable standing surface reduces lameness (Bernardi et al., 2009).

At our research farm we have been able to do controlled experiments, showing how specific management and design features can affect comfort, injuries and lameness, but this research does not show if these same factors will improve conditions on commercial farms that face different challenges. To better describe how different factors affect these outcomes on commercial farms we began a program of on-farm research, initially focused on farms in our region (the lower Fraser Valley of British Columbia; Ito et al., 2009; Ito et al., 2010) and then moving to regions within the United States (California and the North Eastern

United States; von Keyserlingk et al., 2012) and China (Chapinal et al., 2014 a), each with different traditions and where farms face different environmental and economic constraints.

Below we review our approach to benchmarking cow comfort on modern commercial dairy farms and how this affects lameness and hock injuries in adult cows.

Lameness

In each of the regions we have examined, we have found that some farms are able to manage lameness remarkably well, with only a small percentage of animals lame, while other farms struggle in controlling lameness, sometime with more half the cows on the farm showing clinical signs of lameness (see von Keyserlingk et al., 2012).

Cows were scored as not lame (gait score of less than 3 on a 1 to 5 scale; Flower & Weary, 2006), clinically lame (gait score greater than or equal to 3), or severely lame (gait score of 4 or 5). Figure 1 shows the results for 121 farms visited. In British Columbia (BC) prevalence of clinical lameness averaged 28%, versus 31% in California (CA) and 55% in the North Eastern states (NE); the prevalence of severe lameness averaged 7% in BC, 4% in CA, and 8% in NE.

Our primary goal in this 'benchmarking' work has been to engage farmers in discussion about lameness. Providing the farmers with data from their own farm, together with averages from other farms in their region, allowed them to identify areas of success and areas where work was still needed. Each farmer was provided a confidential report that they could use (ideally together with their farm staff, veterinarian, hoof trimmer and nutritionist) to develop tailored solutions for their own farm.

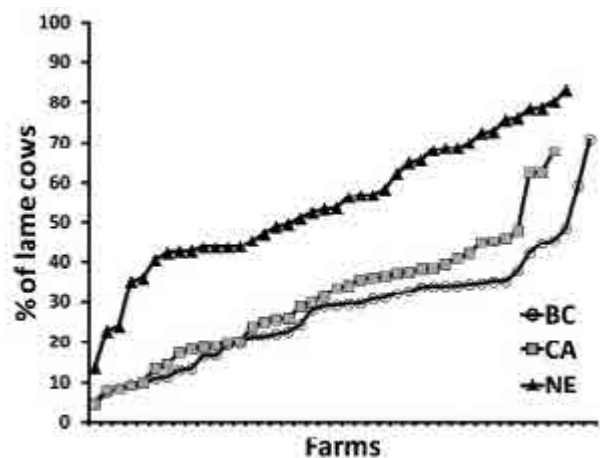


Figure 1. Clinical lameness in British Columbia (BC), California (CA) and the North Eastern US (NE); farms are ranked lowest to highest (from von Keyserlingk et al., 2012).

A second aim of our benchmarking work was to identify housing and management options that lead to lower rates of lameness (Chapinal et al., 2013). Variation in lameness can be explained in part by how barns are designed and managed, but these factors vary among regions due to differences in tradition, barn builders, and availability of materials and bedding. This means that the factors associated with lameness also vary among regions.

For example, in BC we found that the mean prevalence of severe lameness was higher on farms where cows were on mattresses (9% of cows severely lame) versus farms that using deep-bedded stalls (4% of cows severely lame). We also found differences in factors associated with lameness between NE and CA. In NE, where many farms used mats or mattresses with little bedding, the odds of lameness were reduced by half on farms using deep bedding and on farms providing access to pasture for dry cows. In CA, all farms we visited used deep-bedded stalls, and

2 Some of the material in this paper was originally crafted for Research Reports published by the UBC Dairy Education and Research Centre
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almost all of these farms provided outdoor access (typically to a well-bedded dry lot). Likely because of these conditions, rates of lameness were much lower in CA than in NE. Within the CA farms, lameness was lowest on farms where stalls were kept clean (i.e. not contaminated with feces) and on farms that used rubber in the alley leading to the milking parlour. These results suggest that when one limiting factor is addressed (e.g. by changing from mattresses to deep bedding) new factors can be identified (such as the benefits of rubber flooring). In summary, the risk factors for lameness are varied and region specific. For this reason we believe that lameness in particular can benefit from benchmarking programs and the tailored solutions these provide.

Hocks

Unlike lameness, hock lesions are obvious to anyone who cares to look. For example, these can be easily assessed when cows are in the milking parlour providing a clear view of the hind legs. Skin injuries on cattle tend to occur on areas that are in contact with elements of housing, with the most common injuries observed on the knees and hocks. These injuries range from a small area of hair loss to open wounds, and are sometimes accompanied by infection and swelling of the joint.

Cows on each farm were scored for hock condition (lateral surface of the tarsal joint) on a 3-point scoring system; where 1 = healthy hock, 2 = bald area on the hock without evident swelling, and 3 = evidently swollen and/or severe injury. We recorded the % of cows scored with a visible hock injury (i.e. score = 2) and % with severe injury (hock scored = 3).

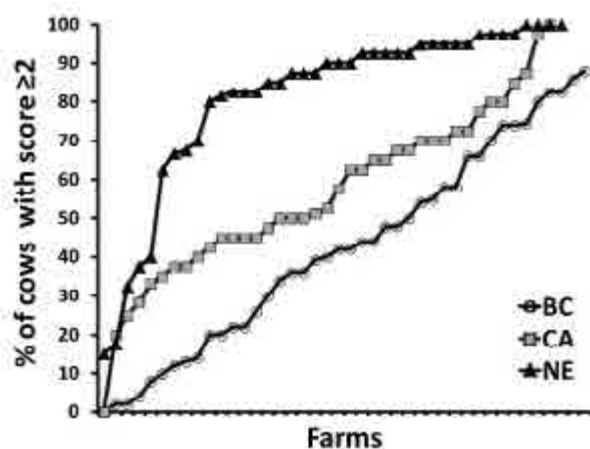


Figure 2. Hock injuries on farms in British Columbia (BC), California (CA) and North Eastern US (NE); farms are ranked from lowest to highest (see von Keyserlingk et al., 2012).

The prevalence of hock injuries varied among regions, from 42% in BC, to 56% in CA, to 81% in NE (Figure 2). The prevalence of severe hock injuries was 4% in BC, 2% in CA and 5% in NE. Our results show that these injuries are all too common, but within each region some producers were able to achieve low levels of hock lesions.

Cows were also recorded for swollen knees (carpal joint); injuries were recorded as present (evidently swollen joint with or without skin damage) or absent. Knee injury was not scored on farms in BC. Swollen knees were rarely observed (less than 1% of cows affected) in CA, but relatively common (23% mean prevalence) in NE.

We know from a series of previous studies, including work in BC more than a decade ago (Weary and Tazskun, 2000), that the risk of hock injuries can be greatly reduced by using deep bedding and that lesions are more common on farms using poorly bedded surfaces like mats and mattresses. This finding helps to explain why lesions were so common

in NE, where poorly bedded surfaces were the norm. Our work in CA and NE has also shown that hock injuries are highly dependent upon bedding management (Barrientos et al., 2013). Most dramatically, in the NE, use of deep-bedded stalls reduced the odds of hock lesions by 95%. Other management practices linked to reduced hock injuries included clean bedding, access to pasture during the dry period, and avoiding the use of automatic scrapers for manure removal. In CA (where all the herds assessed had access to deep bedding) hock injuries were far less common, and farms with well-maintained stalls (i.e. bedding level in the stalls) had the lowest rates. Thus across regions, farms that use well-maintained, deep-bedded stalls had fewer cows with hock injuries. Access to deep-bedded stalls and well-bedded outdoor dry lots also likely explains the low prevalence of cows with swollen knees in CA versus the NE.

Benchmarking programs, like the one we described here, allow producers to compare their performance with averages in their region and provides a basis for informed discussions with professionals involved in farm management, including the herd veterinarian. In the case of hock injuries one solution is obvious – the risk of hock injuries can be much reduced by using deep-bedded stalls.

But does it work?

The ultimate aim of this work is that farmers change conditions and practices to address the problems being benchmarked. We have now begun work to assess how this engagement translates to improved outcomes for the cows. In one recent study, we returned to farms in the NE region of the United States that had been benchmarked approximately 12 months previously (Chapinal et al., 2014 b). This was a convenience sample, consisting of those farmers who were most motivated to have us return to do a re-assessment. Often this was because they had changed housing or management in some way that they perceived would be helpful, so their responses should not be taken as representative. However, among these farms the improvement was impressive. Lameness rates improved on 13 of the 15 farms included in this study, with prevalence often reducing by 10% or more (Figure 3). More impressive still was the improvement in hock lesions; all but one farm improved in some cases hock lesions were essentially eliminated on the farm. Some of the variation among farms in how much improvement was achieved was likely due to variation in how well the changes targeted the limiting factors for that farm. Thus we suggest that the benchmarking process be considered iterative, allowing for change followed by re-assessment, followed by new changes, etc. In this way benchmarking can facilitate a continuous cycle of evaluation and improvements, allowing farmers to judge how well new practices meet their management goals.

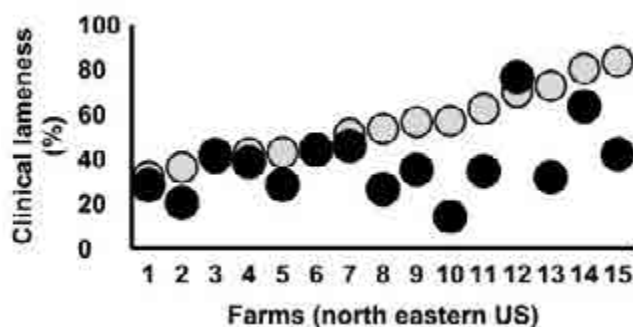


Figure 3. Prevalence (%) of clinical lameness in 15 freestall herds in Northeastern United States in two consecutive farm assessments. Grey and black circles designate the first and second assessment, respectively. Farms are sorted by prevalence at the first assessment (see Chapinal et al., 2014 a).

We believe that that our benchmarking of cow lameness and hock injuries has been successful in getting farmers interested in these

issues, in facilitating the involvement of the herd veterinarian and others into discussions with the farmer on how to address the problems, and at least in some cases leading to real improvements for the cows. In new work we are working to assess these aspects more rigorously, including interviews with farmers and veterinarians, and systematically returning to farms to re-assess outcomes. Our new work is focusing on issues relating to dairy calves, including the perennial issue of failure of passive transfer. We hope to report on our most recent findings during our presentation.

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Science For A Better Life

Dynamics in animal health on beef and dairy cattle

Dr. Calvin W. Booker and Prof. Ynte H. Schukken

Key Speakers

Ynte Schukken D.V.M., M.Sc., Ph.D., Prof.
Chief Scientific Officer GD Animal Health, Deventer, the Netherlands.
y.schukken@gddiergezondheid.nl>

Calvin Booker D.V.M., M.Sc.
Feedlot Health Co.. Alberta, Canada calvinb@feedlothealth.com

Introduction

To feed the growing population – about two billion more people in 2050-animal protein supply has to be increased. The number of animals will not grow at the same rate, but milk and derivatives as also meat will be needed to fulfil the demand. As a consequence more productive cattle is needed and the herds size will increase, predisposing factors for disease spread will be a challenge for Veterinarians.

Objective

The objective of this symposium is to exchange with cattle professionals views on the latest developments with advanced thinkers and doers in cattle science and practice.

Dairy

Global changes in climate, economics, societal pressure, demographics and available resources will have an impact on health management on dairy farms. Fewer farms with a generally less experienced workforce will need to produce more milk for a growing population. These large and growing dairy farms will need to be ready for global climate change causing a change of ecological niche of infectious diseases present on dairies, while societal pressure will result in a reduction of antibiotic use and an increase in concerns on animal welfare. Finally, a reduction in arable land, due to an expanding need for housing and industry, that is available for agriculture will require a greater efficiency of production. Health management of farms will therefore be in continuous transition and veterinary input of these farms will need to adapt to these changes.

Beef

In the case of beef although beef feedlot operations have become more sophisticated in managing health problems, significant economic losses from BRD continue to be related to morbidity and mortality rates, reduced feedlot performance, and metaphylactic and therapeutic regimen costs. The use of simple or complex descriptive epidemiology to study and monitor patterns of disease, as well as data mining and statistical investigations to identify and quantify the impact of potential risk factors, are critical for generating new ideas or hypotheses for potential disease control/mitigation strategies. Subsequently, large-scale clinical trials can be successfully used to evaluate the biologic impact of these strategies on animal health, feedlot performance, and carcass characteristics so that the overall economic impact of each strategy can be determined.

Conclusion

As a consequence of increasing animal protein demand herd size will increase. Diseases in cattle are changing and will be affected by the herd sizes, high production and environmental factors. The latest insights on the influence on cattle health are shared in this symposium.



Make BVD History

Key Speakers

Volker Moennig - Professor emeritus, Institute of Virology, University of Veterinary Medicine, Hannover: Historical aspects on BVD vaccination

Christian Guidarini - DVM Phd Global Technical Manager Biologicals Boehringer Ingelheim: Bovela: time for heroes

James A. Roth - DVM, PhD, DACVM, Center for Food Security and Public Health, Institute for International Cooperation in Animal Biologics, College of Veterinary Medicine | Iowa State University: Humoral and cellular immunity to BVDV vaccination and role in protection

Further information to follow in a separate leaflet in the delegate bag



Elanco Immunity Side Symposium

Hosted by Elanco Animal Health
 Represented by **Mr. Mike Steele** BSc(Hons), BVSc, MRCVS
 of Lilly House, Priestley Road, Basingstoke, UK.
 steele_michael@elanco.com, +44 (0)7712 889298

Key Speakers

Prof. Hans-Joachim Schuberth,
 Hannover Veterinary School, Germany

Prof. Dirk Werling,
 Royal Veterinary College, UK

Prof. Martin Sheldon,
 Swansea Medical School

Prof. Michael Overton,
 Elanco Dairy Analyst

Dr. Peter Canning,
 Senior Research Consultant, Elanco Animal Health

Abstract

A crucial period in the management of dairy cows may be described from dry off to early lactation. This series of short talks focuses on the reduction in function of the innate immune system that occurs in the weeks surrounding calving and its consequences (Kehrli M.E. 1999). Elanco Animal Health have launched an innovative cytokine, to address this reduction in innate immune function. Bovine granulocyte colony stimulating factor (bGCSF) acts to restore the function and increase the number of neutrophils when administered the week before, and at calving. This unique mode of action has been shown to reduce clinical mastitis in early lactation (Kimura K. 2014). This symposium will address the consequences of immune suppression in both the udder and the uterus and allows the audience an opportunity for discussion. The short talks will be introducing the functions and interactions of the innate immune system at calving, the role of the innate system in the endometrium and an analysis of field data regarding key parameters related to immune function, such as mastitis, retained placenta and metritis.

Works Cited

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Early BiRD gets the germs

Key Speakers

David Homer,
Global Head of Ruminant and Equine Technical Services

Sandro Cavirani,
President of the Degree Course in Veterinary Medicine at the University of Parma, ITALY

Claude Hamers,
Senior Veterinary Scientist, Clinical Research & Development

Objectives

Bovine Respiratory Disease (BRD) is a complicated and economically significant pathogen or “germ” challenge for producers, clinicians and the animal health industry as a whole. The solution to better management of it involves a comprehensive herd or population approach, with vaccination programs in young calves (the early bird) a key component. Sounds logical and self-evident but usage of the right antigens at the right time in the right calf vaccination program is not easy to achieve.

Materials & Methods

The symposium will initially briefly cover some of the underlying microbiological, husbandry and anatomical precursors to BRD as well as highlighting why vaccines should be a key pillar of control even though their benefit is sometimes in question. The bulk of the discussion will be around the concepts of herd immunity, pathogen targeting and appropriate vaccination approaches for calves. Pathogen epidemiology, bovine immunology, the role of maternally derived antibodies and vaccinology principles will all be covered in this review. Microbiological and other data from Europe will be used to emphasise a number of the principles. Finally, a new multivalent vaccine (Bovalto) will be briefly presented as an option to consider in vaccination programs for BRD.

Results & Conclusions

It is clear that a high proportion of young calves need to be vaccinated, passive transfer failure needs to be considered in programs and regular reviewing of the pathogen challenge mix is required if an effective herd health approach is to be used. It is also possible that the timetable of vaccination needs to be reconsidered in some circumstances. Finally, it is proposed that it may be time for Buiatrics to consider at least some BRD pathogens as core vaccination targets in both dairy and beef cattle.



The Value of Disease Prevention 'Starts Here'

Hosted by

Fabio Paganini.

Global Head of Food Producing Animals MSD Animal Health

Key Speakers

Mr Didier Moreau,

Director of Environment and Milk Quality of the Danone Dairy WorldWide Division.

Frank O'Sullivan,

Veterinary Surgeon, Patrick Farrelly & Partners

Jeroen van de Ven,

Global Ruminants Lead MSD Animal Health

The Value of Disease Prevention for Milk Processors

Frank O'Sullivan

Implementing Preventive Health programs on farm

Jeroen van de Ven

MSD programs supporting disease prevention

MSD Animal Health will host a symposium titled The Value of Disease Prevention 'Starts Here' Wednesday, July 6th at 17:00 in auditorium. The symposium will feature presentations by guest speakers from Danone and the Irish veterinary profession. Didier Moreau, Global Director for milk quality at Danone and Frank O'Sullivan cattle practitioner and thought leader in the field of preventive health in cattle will present and discuss how averting disease creates value to dairy product customers and the importance of having a strategic approach to an on-farm prevention program.

The final speaker, Jeroen van de Ven, head of MSD Animal Health's global ruminants business, will explain the role MSD Animal Health plays in helping develop new approaches to disease prevention on farms around the world.

We invite everyone to join us at this symposium to engage in this important subject during a time in our industry when, the way we manage the health of animals, is under increasing scrutiny.

Prevention
STARTS HERE 

ORAL PRESENTATIONS

TOPIC

Abortion/Stillbirths
Anti-Microbial Resistance
Beef Herd Health Management
Bovine Welfare
BVD
Cattle Health Economics
Complementary Medicine
Dairy Herd Health Management
Diagnostic Imaging
Education
Emerging Diseases
Epidemiology
Genetics & Breeding
Immunology & Vaccines
Internal Medicine
Lameness
Mastitis Control Programmes
Metabolic Disorders
National Animal Health Programmes
Nutrition
Parasitology
Paratuberculosis
Public Health
Reproduction
Small Ruminants
Surgery
Sustainable Agriculture
Therapeutics
Tuberculosis
Udder Health
Young Stock

AS-050-001

Detection of *Chlamydia* spp and *Chlamydia*-like organisms in placental tissue of bovine abortions in Flanders

Hans Van Loo¹ *Bart Pardon² Marcella Mori³ Daisy Vanrompay⁴ Luc Vanholme⁵ Piet Deprez²

¹DGZ Vlaanderen, Drongen, ²Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, Ghent, ³Veterinary and Agrochemical Research Centre, Brussels, ⁴Department of Animal Production, Ghent University, Ghent, ⁵Federal Agency for the Safety of the Food Chain, Brussels, Belgium

Objectives: To monitor brucellosis in Belgium, farmers are required to report each ruminant abortion to the Federal Agency for the Safety of the Food Chain (FASFC). Besides brucellosis monitoring, the abortion program concurrently screens the submitted samples for several infectious pathogens. Despite the extensive number of analyses included in this abortion program, about 60% of cases remain undiagnosed. One of the possible explanations could be the presence of unidentified abortifacients like *Chlamydia* spp (CS) and *Chlamydia*-like organisms (CLO). The aim of this study was to evaluate bovine abortions in Flanders for the presence of CS and CLO.

Materials and Methods: Between August and November 2013, placentas of 163 late term aborted and 42 healthy calved control cows were collected and analysed using a *pan-Chlamydia* PCR, a *Chlamydia abortus* PCR and a *Parachlamydia acanthamoebae* PCR. CS positive samples were subsequently reanalysed with a *Chlamydia psittaci* PCR. Histological examination was performed on each placenta sample. All abortion cases were also tested for brucellosis by *Brucella* spp culture on the placenta and serology on maternal serum, for *Neospora caninum* by analysis for antibodies on maternal serum, for bovine viral diarrhoea virus by Ag ELISA on fetal spleen and for *Coxiella burnetii* by PCR on fetal abomasal tissue. The fetus was assessed by aerobic bacteriological culture, *Listeria* spp culture and yeast/fungal culture on abomasal and lung tissue.

Results: None of the samples were positive for *C. abortus*. Ten of 163 (6.13%) abortion placentas were positive for CS of which 3 (1.84%) were identified as *C. psittaci*. Seventy-two (44.17%) abortion placenta samples appeared PCR positive for *P. acanthamoebae*. Of the non-abortion control placentas, 1 of 42 (2.38%) sample tested positive for CS (not identified as *C. psittaci* positive), and 4 (9.52%) samples were *P. acanthamoebae* positive.

In 72 of 163 (44.17%) abortion cases, it was possible to detect a potential infectious abortifacient pathogen, different from CS or CLO. In the 91 non-diagnosed abortions, 6 (6.59%) cases were CS positive while 43 (47.25%) cases were *P. acanthamoebae* positive.

Thirty-nine (23.93%) abortion placenta samples were affected by a purulent (necrotic) placentitis, whereas only 2 (4.76%) non-abortion placenta samples were diagnosed with such a placentitis. Twenty of the 82 (24.39%) CS or CLO positive samples showed to have a purulent (necrotic) placentitis.

Conclusions: The detection of CS and especially CLO in a substantial number of bovine abortions, combined with the indicative histological lesions, suggests a potential role of these pathogens in (un)diagnosed cases of bovine abortion in Flanders. Because of the potential risk for zoonotic transmission of CS and CLO, further research is advisable.

Project financially supported by the FASFC and Sanitary Fund.

AS-050-002

Lethal chondrodysplasia in a family of Holstein cattle is due to a *de novo* mutation in the COL2A1 gene

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Vidhya Jagannathan² Cord Drögemüller²

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Objectives: Lethal chondrodysplasia is a well-known congenital syndrome in cattle and occurs sporadically in many breeds. A genetic aetiology has been suspected in many cases, but causal mutations have not been identified until recently. However, chondrodysplasia still remains a puzzle, e.g. cases have occurred among the offspring of a French Holstein sire without significant inbreeding. Genomic analysis of those cases revealed that the mosaic sire transmitted a dominant acting missense *COL2A1* mutation. In 2015, the Danish Holstein sire VH Cadiz Captivo produced several cases of chondrodysplastic calves. Here we report the investigation of these cases.

Materials and Methods: Three malformed calves were submitted and examined by necropsy, histopathology, radiology and computed tomography scanning. Tissue was obtained from additional two cases. Breeding data were analysed based on centrally registered calving data and a questionnaire sent to the breeders. To map candidate regions for the chondrodysplastic condition, genomic DNA of four affected calves, their dams and the sire was used for genotyping of 777,962 SNP markers. Subsequently genetic linkage analysis was carried out. For mutation analysis the whole genome of one affected calf was sequenced using next-generation sequencing technology. Finally, the identified candidate mutation was genotyped using genomic DNA of all five cases, their dams, and the sire by direct sequencing of a targeted PCR product using standard Sanger sequencing.

Results: The calves were morphologically similar and displayed severe disproportionate dwarfism ("bulldog" syndrome) and reduced body weight. The syndrome was briefly characterized by shortening and compression of the body due to reduced length of the spine and the long bones of the limbs. The vicerocranium had severe dysplasia and palatoschisis. The bones had small irregular diaphyses and enlarged epiphyses consisting only of chondroid tissue.

Analysis of breeding data showed that 12.4% (64/515) of the offspring of the sire had chondrodysplasia. Genetic analysis revealing significant linkage to several regions of the bovine genome including chromosome 5 where sequencing revealed a point mutation (g.32473300 G>A) affecting the *COL2A1* gene, which represents a striking candidate gene for bovine chondrodysplasia. The *COL2A1* mutation was predicted to affect splicing because it altered the conserved splice donor sequence GT at the 5'-end of intron 36, which was changed to AT. All five cases carried the mutant allele in heterozygous state and all five dams were wild type GG. The sire VH Cadiz Captivo was shown to be a gonadal and somatic mosaic as assessed by the presence of the mutant allele at levels of about 5% in peripheral blood and 15% in semen.

Conclusions: The lesions were consistent with chondrodysplasia. Genomic analyses identified a causative spontaneous dominant acting mutation in *COL2A1*, which must have occurred during the early foetal development of the sire. Germline mosaicism is a relatively frequent mechanism in the origin of genetic disorders and explains the prevalence of a certain fraction of affected offspring. Paternal dominant *de novo* mutations are a risk in cattle breeding, especially because the ratio of defective offspring may be very high and be associated with significant animal welfare problems.

AS-050-003

Are Low Hepatic Mineral Concentrations Contributing To Aborted, Stillborn And Weak Neonatal Calves?

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Objectives: Fetal abortion and stillbirth are significant pregnancy wastage events contributing to low reproductive efficiency in dairy and beef cattle. Early calf mortalities further contribute to overall reproductive losses. Unfortunately definitive diagnostic outcomes for abortion or stillbirth submissions continue to be less than desired resulting in unknown etiology for more than 30-40% of cases. The study objective was to determine if hepatic trace mineral concentration differences were present among abattoir, aborted, stillborn and neonatal mortality calves in suggesting a potential role for mineral nutrition in perinatal calf losses.

Materials and Methods: Hepatic mineral concentrations (n=382) were retrospectively compared across various populations of fetal and neonatal calves, which died during gestation (n=106), parturition (n=27) or postnatally (n=64; within 2 months of birth) and had been submitted for diagnostic analysis. A population of fetal livers were collected from an abattoir (n=185) were used as "healthy" control comparisons. All liver mineral concentrations were determined by the same university laboratory by inductively coupled plasma spectroscopy (ICP/MS) methods. Mineral concentrations were determined on a wet weight (WW) basis and converted to dry weight (DW) basis using determined liver dry matter content. Measured minerals included calcium (Ca), cobalt (Co), iron (Fe), magnesium (Mg), manganese (Mn), selenium (Se) and zinc (Zn). Abortion and neonatal death submissions were categorized based on laboratory diagnosis as either dystocia (n=35), infectious (n= 36 fetal; 7 stillborn; 40 neonate) or idiopathic (n=35 fetal; 20 stillborn; 24 neonate). Population source (abattoir, abortion, stillborn, neonate), gender, age, breed and other descriptive information were collected. Analysis of variance (ANOVA) models were used to determine main effects of source, disease status, breed (dairy, beef), age (fetal, neonate) and all interactions on hepatic mineral concentration. Orthogonal contrasts were made to evaluate differences in hepatic mineral concentrations based on differing study populations and disease status. Breed was not equally distributed across all populations and was used as a covariate to adjust means.

Results: Across all samples and accounting for breed but not disease status, abattoir samples had greater (P<.0001; .06 for Fe; .003 for Se) concentrations of most minerals, except Ca and Co where they were lower (P<.0001) compared to abortion and stillborn sources. Neonatal samples had lower (P<.054, .018 and .002) Se, Fe and Zn; higher (P<.07, .0001, .0001, and .0001) Ca, Co, Mn and Mo; and were not different in Cu or Mg concentrations compared to abattoir samples, respectively. Breed influenced Se (P<.0001), Ca (P=.04), Cu (P=.002), Fe (P=.03) and Zn (P=.04) with dairy having higher concentrations compared to beef calves for all minerals, except Fe. Disease status influenced (P<.0001) Ca, Cu, Mg, and Co (P=.07), Mo (P=.08) and Zn (P=.01). A source by disease status interaction influenced Cu (P=.0008), Fe (P=.09) and Mn (P=.0065). A source by breed interaction was seen with Ca (P=.0002), Cu (<.0001) and Zn (P=.05). Stillborn calves not caused by dystocia had lower liver Cu (304.0 vs 454.6 µg/g DW; P=.0038), Co (0.066 vs. 0.44 µg/g DW; P<.0001), Mg (500.6 vs. 612.7 µg/g DW; P=.012) and Zn (376.0 vs. 754.1 µg/g DW; P=.0049) compared to those due to dystocia. A number of mineral concentrations, primarily Cu, Ca, Mg, Mn, Zn and Mo in abattoir samples were different from abortion, stillbirths and to a lesser extent neonatal samples. Idiopathic neonatal deaths had lower Mn (6.3 vs. 10.0 µg/g DW) and higher Cu (477.4 vs. 360.4 µg/g DW) and Fe (1102.0 vs. 629.1 µg/g DW) concentrations compared to infectious losses.

Conclusions: Observed differences in hepatic mineral concentrations between abattoir, abortion, stillborn and neonatal mortality calves are suggestive of mineral status being critical to the survival of the fetus during gestation and perinatal period. Trace minerals are involved in a wide range of metabolic functions, including immune function and antioxidant status, which could underpin viability of fetus or calf during the perinatal period. This study's results do not confirm a direct cause and effect, but provide a possible connection that requires further controlled research to confirm the association.

Comments: Research funded by the Pennsylvania Department of Agriculture.

AS-050-004

Bovine Leptospirosis, genotyping and serological diagnosis of bovine abortion cases associated with jaundice in the fetus

Laurent Delooz^{1,*}, Guy Czaplicki¹, Fabien Gregoire¹, Fabiana Dal Pozzo², Floriane Pez³, Angeli Kodjo⁴, Claude Saegerman²¹Santé animale, ARSIA asbl, CINEY, ²UREAR, ULg - FMV, Liège, Belgium, ³BioSellal, ⁴Laboratoire des Leptospores, VetAgro Sup, Lyon, France

Objectives: Leptospirosis is a global disease with potential major economic impact on livestock industries and important zoonotic capacities. The disease surveillance in the developing economies represents a major challenge as humans and animals frequently live in close association such as veterinary and farmers. The serovar Hardjo of *Leptospira* whose primary host is cattle has been studied extensively, but few data exist on other current circulating or emerging serovars. To better understand the disease in cattle and how to prevent and/or control it, it is necessary to identify the genotype and the serovar of circulating *Leptospira*.

Materials and Methods: Between January 2009 to December 2014, 5368 herds submitted to the laboratory 18,014 cases of bovine abortions including at least a foetus. Samples were obtained in the context of the passive surveillance program for brucellosis and were collected by the local veterinary practitioner within 48h after abortion and sent to the regional laboratory of southern Belgium (ARSIA) together with an anamnesis. Sera of the dam were tested using the standardized panel of analyses and were subsequently stored at -20°C. An autopsy and a standardized panel of analyses were performed on organs that were subsequently stored at -20°C. In addition, a *Leptospira interrogans* spp. RT-PCR (ThermoFisher, France), a *Leptospira* genotyping method by multilocus sequence typing (Biosellal, France) and a microscopic agglutination tests (Laboratoire des Leptospores, VetAgro Sup, France) were subsequently performed on some samples.

Results: This study presents results of several investigations performed on a historical Belgian collection of congenital jaundice in bovine aborted fetus cases in order to identify and characterize its *Leptospira* etiology. The serovar and the genotype of two pathogenic *Leptospira* strains were determined, i.e. *Leptospira kirscheneri* Grippotyphosa serogroup and *Leptospira interrogans* Australis serogroup. In addition, microscopic agglutination tests revealed also that serogroups Grippotyphosa and Australis were the most prevalent with respectively 17/42 and 13/42 positive MAT. To identify a specific seroconversion against leptospiral serogroup, a paired serology with an average interval of 60 days between samplings was performed after abortion on twenty dams. Seroconversion was only observed for Australis serovar on three aborted dams.

Conclusions: In conclusion, this study allows the association of two pathogenic *Leptospira* strains to congenital jaundice in bovine aborted fetuses. As a consequence, this clinical sign should be considered as an additional clinical picture of bovine leptospirosis. Considering the difficulty to diagnose this disease among infected cattle, the diagnostic approach established by ARSIA seems to be an efficient method under field conditions in which the veterinary practitioner performs samples in cattle farms.

AS-050-005

An outbreak of congenital chondrodystrophy of unknown origin (CCUO) among commercial beef calves in central Alberta

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Objectives: An outbreak of congenital joint laxity in the presence or absence of chondrodystrophy occurred at a commercial cow-calf operation in central Alberta during spring of 2014. All affected calves were born to home-raised crossbred heifers and sired by a single Angus bull. There was no history of feeding silage, which has previously been associated with outbreaks in Canada. The objective was to investigate other etiologies of congenital chondrodystrophy.

Materials and Methods: During mid-November 2013 bred heifers were moved from summer grazing pasture to winter pasture with well-sourced water. The producer reported feeding approximately 16.8kg of grass hay (chopped round bales) and 1.4kg of barley per head per day. They were supplemented with varying brands of commercial trace mineral and salt with added selenium (approximately 20kg every 1.5 to 2 weeks.) The mature cow herd was pastured separately and received the same source hay and mineral but no barley. The winter was notably harsher than usual with increased snowfall and below average temperatures.

Heifers began calving March 11 extending to May 15, 2014. During that time 29 of 32 calves born were affected with congenital joint laxity in the presence or absence of chondrodystrophy. There were no apparent trends in calving dates versus severity of abnormalities. However, it is noteworthy that 1 of the 3 normal appearing calves was born to a heifer mistakenly overwintered with the mature cow herd. Cows began calving on March 15 extending to May 21, 2014 and gave birth to all normal appearing calves.

The majority of heifers were assisted in delivery and 5 calves were stillborn. Multiple calves died or were euthanized as a result of complications related to failure to ambulate or thrive after several days of supportive care. Joint laxity resolved in surviving calves by 2 weeks of age. Thirteen of 32 calves born to heifer dams survived until weaning. Residual abnormalities in affected calves included chondrodystrophy, superior brachygnathia, and multiple angular limb deformities.

Results: Five affected calves were necropsied by a board certified pathologist and chondrodystrophy was confirmed in 4 by metacarpal length/diameter ratio. Other than obvious congenital abnormalities, physical examinations of both live and dead affected calves were unremarkable. All heifer dams were normal appearing moderate-framed crossbred heifers with body condition scores at calving ranging between 4 and 5 on a 9-point scale.

The sole sire, along with 4 heifer dams giving birth to affected calves and 2 affected calves were tested for genetic abnormalities. All animals tested negative for carrier status of arthrogyposis multiplex, contractural arachnodactyly, and osteopetrosis. Serum mineral concentration was also analyzed in the 4 heifer dams. Minerals outside of normal range included: high normal molybdenum (3 head), marginal magnesium (2 head), marginal copper (1 head), and deficient copper (1 head). Unfortunately feed was no longer available to be tested for nutrient composition, mold or toxins.

Conclusions: In agreement with several previously reported outbreaks of congenital chondrodystrophy of unknown origin worldwide, this outbreak was associated with spring calving heifers. Genetic causes have been deemed unlikely and a nutritional-related issue, particularly trace mineral deficiency is highly suspected. Lack of feed analyses and imprecise records precluded accurate calculation of estimated daily intakes for minerals critical for normal epiphyseal growth plate formation such as manganese. In addition, the high-risk period (3-6mo. gestation) overlapped with summer grazing and winter pasture time frames further complicating investigation.

AR-037-001

What do UK vets' think about the efficacy of teat sealants versus antibiotics for preventing infections? How does new evidence alter their beliefs?

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Objectives: The existing evidence suggests that in uninfected quarters at dry-off, using a teat sealant (TS) instead of an antibiotic (AB) is as effective at preventing new intra-mammary infections (IMI). Changing from an AB to a TS can considerably reduce antibiotic use. The aims were:-

1. To quantify vets' current beliefs regarding the efficacy of an AB versus a TS at preventing new IMI in uninfected quarters at dry-off.
2. To quantify how vets' update their clinical beliefs when presented with new data that challenges their current beliefs.
3. To assess how logically vets alter their beliefs in the light of new evidence.

Materials and Methods: In total, 20 face-to-face interviews were conducted with UK farm vets located in six practices in the South of England. Probabilistic elicitation (roulette method) was used to capture the vets' current beliefs as probability distributions (called the "prior distributions"). Thereafter, the vets were shown new data that challenged their current beliefs. The vets' beliefs were re-captured as probability distributions in light of the new information (called the "posterior distributions"). We assessed how logically the vets' updated their beliefs by comparing how they changed to a gold standard - predictions from Bayes theorem.

Results: For 17 vets, the mode of their prior distribution (i.e. their "best guess") indicated that a TS was superior to an AB at preventing new IMI. However, there was clinical ambiguity, such that 12 out of the 20 vets assigned some probability that the AB was the superior treatment, with the remaining 8 vets giving negligible probability to this event. There was also heterogeneity in the magnitude of the difference in the treatment efficacy expected between the two treatments.

In total, 4 out of the 20 vets updated their beliefs perfectly logically in light of the new data (both central location and variance). Furthermore, the majority (18/20) centred their posterior distributions correctly. However, the majority (16/20) did not adjust the variance of their posterior distributions in keeping with Bayesian predictions. Specifically: 8 vets had posterior distributions with variance less than their prior but greater than predicted, 6 had posterior distributions with variances equal to their prior, and 2 had posterior distributions with variances greater than their prior. Thus, many vets did not draw as much confidence from the new data as could logically be expected.

Conclusions: Results suggest that some vets are not entirely convinced of the efficacy of teat sealants. This has important implications for responsible antimicrobial prescribing, especially given there are many potential challenges with changing from AB to a TS.

Evidence-based practice relies heavily on changing beliefs by presenting new data. Yet people do not always respond logically when shown new data, especially if it contradicts their pre-existing beliefs. Our results suggest that some vets may benefit from additional training to help them update their beliefs appropriately and to facilitate the efficient transfer of new evidence into practice.

AR-037-002

Developing a multifaceted, collaborative, practice-wide approach to responsible medicines use on farms

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Objectives: Langford Farm Animal Practice (LFAP), a clinical teaching practice of the University of Bristol, has a long history and developed culture of proactively engaging with farm clients to encourage responsible antimicrobial use. The objective of this ongoing project is to demonstrate that farm animal veterinary surgeons in a commercial practice, working in partnership with farmers, can minimise the risk of antimicrobial resistance (AMR) development without compromising animal health and welfare, by reducing total and critically important antimicrobial use on farm through improved herd health management (HHM) and systematic medicines auditing.

Materials and Methods: LFAP serves 220 farms in Somerset, UK, representing approximately 1500 dairy cows with an average herd size of 150; 3300 beef cattle and a similar number of sheep, with smaller numbers of other species. A multifaceted, practice-wide, collaborative approach combining changes to HHM and prescribing policy with systematic medicines audits and farmer training was adopted. Farmers' meetings and newsletter articles raised awareness, encouraged engagement and provided training on compliance and responsible use.

Beginning in 2009, fluoroquinolone use was halted and critically important antimicrobials progressively withdrawn from first-line treatments. Fourth-generation cephalosporins were removed, and third-generation cephalosporins replaced with first-generation cephalosporins or aminopenicillins. Calf pneumonia cases were treated with oxytetracycline or florfenicol, as an alternative to longer-acting macrolides.

Farm- and practice-level medicines audits were introduced in 2012 as part of HHM and clinical governance, respectively, using a bespoke Excel (Microsoft Office 2010) spreadsheet. An annual retrospective analysis of medicines use data at practice level from 2010 onwards was completed. Multiple measures were used for benchmarking, including animal daily doses at farm level and total milligrams per year at practice level, due to the broad species demographic. Consideration of both animal health and economic implications of current medicines use, including the balance between preventive interventions and reactive treatments, allowed targeted recommendations to be made.

Results: Unless otherwise stated, at practice level, total milligrams of antimicrobials used is reported.

No fluoroquinolones have been prescribed since 2009. Respectively, 89.8% and 63.5% reductions in the systemic use of third/fourth-generation cephalosporins and macrolides were achieved. Macrolides now account for less than 0.1% of antimicrobial treatments for pneumonia. Intra-mammary use of fourth-generation cephalosporins in the treatment of mastitis was reduced by 99.7%. These now account for 0.2% of lactating cow intra-mammary tubes, compared to 32.7% in 2010.

Overall this equates to an 87.3% reduction in the use of fluoroquinolones, third/fourth generation cephalosporins and long-acting macrolides, compared to 2010 levels. Total antimicrobial use in milligrams increased by 5% over the same period, against a 10% increase in dairy cattle numbers and an overall reduction in the number of courses prescribed. There was no evidence of declining herd health or cure rates (whether real or farmer-perceived) with a move to first-line treatments.

Farm clients have been consistently positive about tackling the issues of responsible medicines use and AMR in partnership with the practice. Concern over farmer willingness to participate was unfounded. Informal

feedback suggests farmers have found measures which express antimicrobial use in terms of doses, courses or economics most useful.

Conclusions: Medicines audits are a useful and effective tool to both monitor and drive responsible antimicrobial use and to reduce total antimicrobial use in food-producing animals. It is likely that the combination of a collaborative approach, which built upon the existing, relationship of trust between farmers and veterinary surgeons, with a focus on improved HHM, contributed to success. For practitioners interested in responding to the threat of AMR, this work demonstrates that marked reductions in the use of critically important antimicrobials can be achieved, whilst keeping farmers engaged and continuing to improve animal health and welfare.

AR-037-003

Antimicrobial resistance in large Swedish dairy herds with high or low calf mortality risk

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Objectives: Calf mortality is reported worldwide as an important cause of economic loss in dairy production. Previous studies have shown that a high calf mortality was associated with higher antimicrobial use. Commensal *Escherichia coli* can acquire resistance genes or develop resistant mutants; these are regarded as indicators of the antimicrobial exposure of their host. In this study we aimed to evaluate if treatment and management regimes at herd level were associated with antibiotic resistance in *E. coli* at individual level and also to evaluate if risk factors for resistance were associated with type of herd (high versus low calf mortality risk).

Materials and Methods: Sixty large dairy herds were enrolled in the study. All were affiliated to the Swedish official milk recording scheme, which covers 90% of all Swedish dairy cows and involves detailed recording of data on management and production. The inclusion criteria were: herd size ≥ 160 cows, calf mortality risk (day 1-90) $\geq 6\%$ (HM-high mortality) or $< 2\%$ (LM-low mortality). The herds were visited once during December 2005 to March 2006 and up to 5 faecal samples were collected per rectum from visually healthy calves 1-14 days of age. Samples from calves which had been treated with antimicrobials were omitted in the statistical analyses. All faecal samples ($n=238$) were streaked onto MacConkey agar plates. Antimicrobial susceptibility testing was carried out for lactose-positive isolates using cut-off values set by the European Committee on Antimicrobial Susceptibility Testing.

Treatment and management variables ($n=21$) were first screened using univariable logistic regression with either antimicrobial resistance (at sample level) as outcome or type of herd (HM/LM; at herd-level). Variables associated with type of herd at $p \leq 0.20$ were qualified for further multivariable analysis. In total, six multivariable models with resistance to different types of substances as outcome were evaluated: resistance to nalidixic acid, sulfamethoxazole, streptomycin, tetracycline, ampicillin and presence (0/1) of multiresistance to ≥ 5 substances.

Results: None of the calves in the analysis had received antimicrobial

treatment. The five substances for which resistance was most frequently shown, were nalidixic acid, sulfamethoxazole, streptomycin, tetracycline and ampicillin corresponding to 65%; 55%; 46%; 42% and 40% resistance. Presence of resistance against these substances were ranked in the same order in HM and LM herds. All herds, except one, showed antimicrobial resistance to at least one substance. Of the 226 samples, 44 samples (24%) were diarrhoeic ($n_{HM}=29$; $n_{LM}=15$). Diarrhoeic samples were significantly more common in high mortality herds ($p=0.012$; OR: 2.4; 95% CI: 1.21–4.80).

Multivariable analyses: The model with nalidixic acid as outcome remained non-significant. All models with resistance as outcome had one thing in common: a diarrhoeic sample was significantly associated with higher odds of resistance. The model with sulfamethoxazole as outcome showed higher odds for resistance for herds starting antimicrobial treatment against pneumonia earlier; when calves were coughing compared starting treatment when calves had fever ($p=0.007$; OR: 10.13; 95% CI:1.86-55.14). Associations with type of herds were demonstrated in the model with multiresistance to ≥ 5 substances, with more multiresistance in HM herds (OR: 2.28; 95% CI:1.15-4.52). Multivariable results from all of the models will be presented.

Conclusions: Antimicrobial resistant bacteria were common in calf faecal samples with loose consistency (diarrhoeic samples). Herds with high calf mortality had higher prevalence of multi-resistance to ≥ 5 substances. Because none of the sampled calves had been treated with antibiotics this indicates other routes of introduction of antimicrobial resistance than treatment of the calves. An excessive use of antimicrobials for early treatment of pneumonia is associated with higher odds for sulfamethoxazole resistance.

AR-037-004

MRSA in organic dairy farms in Germany – is it an issue?

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Objectives: Antimicrobial resistance (AMR) is currently one of the major public health issues. Public perception relates AMR rather to conventional than organic farming. The objective of this study was to compare the prevalence of methicillin-resistant *Staphylococcus aureus* between bulk tank milk (BTM) from organic and conventional dairy farms in Germany.

Materials and Methods: Sampling was carried out in 2014 and distributed across the country based on the regional distribution of conventional and organic dairy cows in Germany. BTM samples (25 ml) were analysed by regional laboratories for MRSA using a double selective enrichment method. Isolates were sent to the National Reference Laboratory for coagulase-positive *Staphylococci* incl. *Staphylococcus aureus* (NRL) for PCR confirmation and typing. Resistance to 19 antimicrobials was tested using the broth microdilution method according to CLSI standards by the National reference laboratory for antimicrobial resistance. Minimum inhibitory concentrations were evaluated according to EUCAST epidemiological cut off values.

Results: Overall, 9.7 and 1.7 % of 372 and 303 analysed samples from conventional and organic dairy farms respectively tested MRSA-positive. From conventional dairy farms, 36 isolates were sent to the NRL for

typing and resistance testing. Five isolates originated from organic farms. Most isolates (34 and 4) had spa-types related to the clonal complex CC398. However, 1 organic and 2 conventional isolates had spa-types related to other clonal complexes, one of those being a typical human associated type (spa-type t790, CC22). Overall MRSA from conventional dairy farms were only slightly more resistant than those from organic farms. However, as only few MRSA were submitted from organic farms no statistical evaluation was valid.

Conclusions: Results indicate that MRSA are less prevalent in organic than in conventional dairy herds in Germany. Compared to similar studies 2009 (4.1 %) and 2010 (4.7 %) the proportion of MRSA positive conventional dairy herds increased substantially. Moreover, non-CC 398 Isolates had not been detected in that study. As *S. aureus* is an important mastitis pathogen in cattle, spread of MRSA is worrisome not only from a public health perspective. Although significantly less frequently organic dairy herds also harbour MRSA.

Comments: The authors are grateful to the regional laboratories performing the primary isolation and to the Federal Office for Consumer Protection for support in data collection.

AR-037-005

Antimicrobial resistance in *E. coli* from organic and conventional dairy herds in Germany

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Objectives: Antimicrobial resistance (AMR) is currently one of the major public health issues. Public perception relates AMR rather to conventional than organic farming. It was the purpose of this study to compare AMR in *E. coli* harvested from bulk tank milk (BTM) in conventional and organic dairy herds in Germany

Materials and Methods: Sampling was carried out in 2014 and distributed across the country based on the regional distribution of conventional and organic dairy cows in Germany. BTM samples (25 ml) were analysed by regional laboratories for *E. coli* and isolates were sent to the National Reference Laboratory for Antimicrobial Resistance at the BfR. Resistance to 14 antimicrobials was tested using the broth microdilution method according to methods described in Commission Decision 2013/652/EC on the harmonized monitoring of AMR in the food chain in the EC. Minimum inhibitory concentrations were evaluated according to EUCAST epidemiological cut off values.

Results: Regional laboratories sent 74 and 122 isolates of *E. coli* from organic and conventional dairy farms respectively. The vast majority of the isolates was susceptible to all 14 test substances. Of the 3 organic isolates (4.1 %) that were resistant to at least one substance, all were resistant to tetracycline, one additionally to ampicillin and another one to tetracycline and sulfamethoxazole. Among the 13 conventional resistant isolates (10.7 %) resistance to ampicillin (7 isolates), sulfamethoxazole (7) and tetracycline (6) was also most prevalent. However, resistance to ciprofloxacin, a fluoroquinolone (2), colistin (1) and ceftazidime, a 3rd generation cephalosporin, (1) were also observed.

Conclusions: Results underline that overall, AMR is not a major issue in dairy herds. However, the proportion of resistant *E. coli* was even lower in organic dairy herds and resistance was only observed to comparatively old antimicrobials with widespread resistance in other

food animal populations. In contrast, isolates from conventional farms showed some diversity in resistance which might reflect differences in selective pressure in the herds.

Comments: The authors are grateful to the regional labs for doing the primary isolation.

AR-037-006

Antimicrobial Use In Dairy Cattle In New Zealand: A National And Regional Benchmarking Study

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Objectives: The New Zealand Veterinary Association (NZVA) has recently set an aspirational goal of using zero antimicrobials for the maintenance of animal health and welfare by 2030. A key strategy in achieving this goal is understanding the current use rates of antimicrobials.

The objective of this study was to measure and understand antimicrobial use and trends in New Zealand dairy cows, and to set a baseline level to facilitate benchmarking and the monitoring of antimicrobial use at a national level. Two databases were utilised, representing cows in Southland only, and a national database representing cows in all regions of New Zealand.

Materials and Methods: Southern regional antimicrobial sales data from dairy clients were extracted from the VetSouth veterinary clinic database (representing approximately 44% of all dairy cows in Southland) for 2013, 2014 and 2015 seasons. In addition, national antimicrobial sales data (2015) was obtained from the WelFarm database (data gathered by 6 participating XLVets veterinary clinics located throughout New Zealand).

The mass of antimicrobial agents sold was calculated by multiplying the volume of product sold by the concentration of each active agent (after accounting for the sales unit). Each active agent was classified by antibiotic class and product type.

The animal biomass (at peak milk, December) was calculated by the multiplication of cow (heifer) numbers by the industry accepted average New Zealand dairy cow (heifer) weight of 460kg (270kg), respectively. If heifer numbers were unavailable for a particular farm, heifer counts were estimated by multiplying the cow number by an estimated average replacement rate of 24%.

Antibiotic sales volume was normalised by dividing the mass of the active ingredients (mg) by the biomass/population correction unit (kg).

Results: A total of 979kg, 1138kg and 1249kg of antimicrobial agents were sold to Southland dairy farmers for 2013, 2014 and 2015, respectively. The total number of cows (heifers) in the dataset were 253,106 (61,652), 259,704 (65,010), and 286,208 (69,293) from 399, 406, 436 farms, for 2013, 2014 and 2015, respectively.

A total of 271kg of antimicrobial agents were sold to 109 dairy clients from 6 regions participating in the WelFarm programme during the 2015 season. The total number of cows (heifers) was 68,337 (16,587), respectively.

Penicillins were the most commonly sold antibiotic by volume (78.0%, 74.4%), followed by macrolides (10.2%, 10.9%), cephalosporins (4.7%, 8.3%) and tetracyclines (4.2%, 4.7%), for VetSouth and WelFarm clients, respectively.

Total annual mg/PCU was 8.31 and 7.57 respectively. The standardised measure of antibiotic use (mg/PCU) for the 2015 season was; aminoglycoside (0.09, 0.09), cephalosporin (0.39, 0.63), quinolone (0.003, 0.003), lincosamide and macrolide (0.85, 0.84), penicillin (6.48, 5.63), sulphonamides and trimethoprim (0.15, 0.02), and tetracycline (0.35, 0.36), for VetSouth and WelFarm clients respectively.

Amongst VetSouth clients, there was a decreasing trend of cephalosporin and macrolide sales (mg/PCU) from 2013 to 2015 (0.71, 0.61, 0.39 and 1.03, 0.90, 0.85, respectively), however there was an increasing trend in penicillin sales (5.01, 6.16, 6.48, respectively).

The most common product type sold (by volume) was injectable products (49.8%, 56.4%), followed by intramammary dry cow (41.6%, 35.1%) for VetSouth and WelFarm clients, respectively.

Conclusions: The data presented sets a baseline level of antimicrobial use in New Zealand. The results show that penicillins are the most commonly used antibiotic in New Zealand dairy cows. Furthermore there appears to be an increasing trend of penicillin use over the last 3 years, whereas cephalosporin and macrolide use has decreased.

These data can also be compared with national –level pan- species antibiotic use data. Data collated from antibiotic sales data has demonstrated an overall annual use rate of between 7.62 and 11.46 mg/PCU for all species. These data demonstrate that dairy cattle fall within the lower- mid range of the overall species data.

AR-037-007

From Policy-Takers To Policy-Makers: Development Of An Antimicrobial Stewardship Policy By Dairy Farmers

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Objectives: Pressures for more responsible use of antimicrobial medicines in food animals are likely to increase from consumer groups, policy-makers and the food industry, including retailers. Participatory approaches to disease prevention and medicine management offer an innovative way to approach these challenges while maintaining or improving dairy herd health and welfare. This research sought to examine the processes involved in engaging and enabling groups of dairy farmers in the conception, design and implementation of an antimicrobial stewardship policy for a major retailer in the United Kingdom.

Materials and Methods: In this project, dairy farmers, cooperative leaders, veterinary surgeons, researchers and facilitators worked together to conceive of and develop an antimicrobial stewardship policy that would meet the needs of the retailer and would be fully enactable on farms, allowing farmers to reduce and rationalise antimicrobial use without compromising animal health and welfare. All farms (n=80) supplying the retailer were invited to participate, along with their veterinarians, in policy-creating meetings led by a facilitator. The process provided a unique opportunity for collaboration and dialogue between farmers, veterinarians, industry and researchers, with farmers taking a leading role in generating feedback and ideas. Information was sought on farmer and veterinarian attitudes towards antimicrobial resistance and antimicrobial use on their farms. Meetings were loosely structured around a conceptual model (Speksnijder et al., 2015), and specifically addressed disease reduction strategies, ensuring correct use of

antimicrobials, avoiding prophylactic use of antimicrobials and ensuring quality data recording and use. Each of these areas was expanded, and farmer's comments were compiled to develop a framework for a policy which was further refined and finalised following farmer feedback. Implementation of the policy is currently on-going and includes measures agreed by all participating farmers.

Results: Attendance at all meetings was exceptional, with over 95% of farms represented. Veterinary response was also high, with engagement of almost all the veterinary practices servicing the supplier farms. Farmers were responsive and aware of the problem of antimicrobial resistance. Farmers reported enjoying the meetings and felt their voices were heard, however, they also expressed that development of such a policy was difficult and mentally taxing. Veterinarians participated extensively and were also active in discussions. After these initial data-gathering meetings, drafting and refinement of the policy was accomplished by a smaller group of industry representatives, farmer representatives and researchers. Further feedback and refinement of the initial policy was then achieved through follow-up meetings with a larger group of farmer representatives. The agreed policy included aims and measures to achieve more responsible use of antimicrobials, and was accepted by all farmers. Once finalised, the policy was reported to all farmers, and implementation begun. Collaboration of the researchers with the supply chain is ongoing to ensure full compliance and continue adapting the stewardship policy as needed.

The participatory nature of this work encouraged comprehensive learning for all involved. This integration of science with farmers' knowledge and experience led to credible and practical recommendations designed to deliver real and lasting change in antimicrobial use.

Conclusions: The multidisciplinary nature of this research contributed to the embedding of social science skills and approaches into the veterinary sphere. As an initial step in creating better understanding of how participatory approaches with farmers can be applied in a UK context and more widely, this work serves as a pilot for promoting more responsible use of veterinary medicines in other livestock species. Feedback was overwhelmingly positive, and engagement of farmers with the process is expected to lead to increased uptake of changes and improved stewardship of antimicrobials.

BH-019-001

An Epidemiological Study Of A Pneumonia Outbreak In An Irish Beef Feedlot

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Objectives: Pneumonia is the most significant animal health issue affecting the beef industry worldwide. While there is quite an amount of international literature on factors predisposing to pneumonia, there are few recent studies on factors predisposing to pneumonia in Irish beef herds. The objective of this study was to study risk factors associated with the occurrence of pneumonia in an Irish beef herd.

Materials and Methods: The study population comprised of a subset of bulls purchased into an Irish feedlot in 2014.

For each animal purchased, data assembled from the Animal Identity and Movement (AIM) database included their ear tag, the herd of origin, age at purchase, age at death, days on the farm, breed, date of purchase, week of purchase, month of purchase, the mart if any through which they were purchased, the date of slaughter. The following data were assembled from the Irish Cattle Breeding Federation (ICBF) database; weight at purchase, carcass weight and grade at slaughter. The farmer provided the pneumonia treatment status. Descriptive statistics were calculated for the dataset, and univariate and multivariate analysis was carried out.

Results: It was found that the odds of pneumonia occurring in November purchased cattle was twice that of cattle purchased in October, which in turn was twice the odds of pneumonia in September purchased animals. Bulls purchased at a younger age were found to have a greater risk of pneumonia. The odds of pneumonia doubled for each 50 day decrease in age. The live weight at slaughter of non affected bulls was on average 13kg heavier than bulls affected by pneumonia. There was no difference in odds between the 15 marts in which the bulls were purchased, or among the 9 breeds of cattle purchased. Younger bulls purchased later in the year had the greatest risk of pneumonia. The performance of animals affected by pneumonia was significantly diminished.

Conclusions: In addition to the costs of treatment and increased labour, pneumonia reduces the performance of beef bulls. In this study the lifetime performance of affected bulls was reduced by 0.1 kg per day. This loss in performance coupled with the costs of treatment and increased labour are a significant loss to bull beef finishers, and emphasises the need for the control of respiratory disease in feedlots. As part of the control strategy, bull beef finishers should consider purchasing older calves earlier in the season.

BH-019-002

Characterization of bioaerosols in beef cow-calf operations over a 6 month period: relations with environmental risk factors and respiratory disease

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Objectives: Bovine Respiratory Disease (BRD) is an important economic problem in cattle industry, and is the most common cause of death for pre-weaning beef calves more than 3 weeks old. BRD is due to complex interactions between pathogens and environmental management factors and traditionally associated with poor ventilated housing conditions. Surprisingly, only few data are available concerning the impact of microbial quality of the air on BRD. The objectives of this study were to analyze relationship between air quality, a variety of environmental risk factors and respiratory health in beef cow-calf operations.

Materials and Methods: A Coriolis air sampling device (Bertin technologies) was used to collect samples in 17 beef cow-calf operations buildings, 9 tie stall and 8 free stall barns, each month over a 6 months period (From December to May). Sampling consisted of 10 minutes aspiration at 250L/min in three different barn areas: calf pen, central alleys (tie stall barns) and/or cow straw bedding (free stall barns). Aerosols were collected in 15 mL PBS tween 0.05%, by high-speed cyclonic motion without filtration. Airborne total bacterial counts (cfu/m³) were determined by direct isolation on Trypticase Soy Agar plates (TSA). Airborne total molds counts (cfu/m³) were obtained after isolation on Sabouraud Gentamicin Chloramphenicol 2 agar plates (SGC2). DNA loads of bacteria, Aspergillus and archeal bacteria were quantified by real-time quantitative PCR assays on a LightCycler 480 (Roche). Dimensions of the barn, sidewalls, alleys, calf pens were measured. Calf pens were assigned a box factor (1 to 3) related to the number of solid planes around the calf. During aerosol sampling, temperature, hygrometry and wind speed were recorded using a KIMO HD200 STD data logger (Kimo). Stocking density (m³/kg) was calculated (for each month and each barn) by dividing the barn volume by the product of total number of calves and their average estimated weight. Prevalence of BRD was determined as the percentage of total number of calves treated for respiratory signs at least one time. Statistical analysis was performed thanks to time series mixed linear or binomial regressions with operation as random effect.

Results: Results show large differences of bacterial and mold counts between farms, ranging from 1.2.10² to 6.8.10⁵ cfu/m³ and 1.2 to 2.3.10⁶ cfu/m³ respectively. A large variability between farms was also observed for DNA loads of bacteria, molds and archeal bacteria in aerosols. DNA loads of bacteria and archeal bacteria were correlated (p<0.001) but surprisingly there was only weak correlation between isolation counts and qPCR. No difference in counts was observed between tie stall and free-stall barns (p>0.05) although temperature within barns was significantly higher in tie stall compared to free-stall barns (p<0.001). Within each barn, airborne content was not dependent on the location of sampling. Despite variation in environmental factors, no significant differences were seen within each barn in microbial air content when animals were present (From December to April). Significant difference was tough observed when cattle were in barns or outside (May). A prevalence of BRD of more than 15 % was associated with increasing boxfactor (OR: 2.8, 95 CI=1.1-7.6), decreasing stocking density, and with use of vaccine (P<0.01).

Conclusions: Development of portable air sampling devices has made it clinically feasible to evaluate air hygiene in livestock buildings. Results suggest a large variability in microbial air content between herds, which is not correlated to tie or free stall systems. Within one farm the microbial air content seemed to remain constant until the animals go outside. Prevalence of BRD is correlated to environmental factors and management practice and could be linked to microbial air content. Further studies on quality of the aerosols (NGS and 16S sequencing of air contents) are in progress to determine its impact on BRD.

BH-019-003

The value of using enhanced case definitions for the management of BRD in commercial feedlots

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Objectives: Bovine respiratory disease (BRD) is the primary cause of morbidity and mortality in cattle during the feeding period. Management of BRD cases relies on the observation of cattle behavioral changes by caregivers. Applying multiple ante-mortem diagnostic technologies in combination improves outcomes in the clinical management of BRD.

Materials and Methods: After being identified as sick and pulled from the home pen, cattle are typically moved to a treatment area, where a case definition (CD) for BRD presence, severity and chronicity is assigned by a trained caregiver, based on rectal temperature (RT), attitude and appearance. We applied a computer-based algorithm (Whisper®, Geissler Corp.) to sounds recorded during the initial assessment for suspected BRD of 17,848 cattle from 13 sites to determine if algorithm-based analysis of lung auscultation would improve BRD CD precision. A discrete score (LS) of 1 to 5 was applied to each calf (1 = normal, 5 = chronic or severe BRD) for use in the treatment decision protocol. RT was also a factor in the treatment protocol. Outcomes were measured by case fatalities (CF). We expected a high LS to be associated with a greater chance of CF. A logistic regression model using BW, RT and LS was constructed to estimate the likelihood of CF.

Results: For each 1 unit rise in LS, the likelihood of CF increased by 49.4% adjusted for RT and BW (odds ratio (OR) = 1.494, 95% CI 1.428-1.564). For each 1 degree F increase in RT, the likelihood of CF increased by 19.1% (OR = 1.191, 95% CI 1.149-1.234). There were no interactions between temperature and LS detected. Treatment (antibiotic) was not associated with different outcomes and there was no interactions between treatment and LS or temperature and therefore was not included in the final model.

Conclusions: These data suggest that algorithm-based analysis of lung auscultation is an effective tool in practical applications to predict individual clinical outcomes. Standard case definitions for BRD based on lung auscultation and rectal temperature can improve evaluation of treatment success and can be used in the development of evidence-based treatments for BRD.

BH-019-004

Effects of castration and castration method on growth, morbidity and mortality in bulls 30-50 days post entry to a feed yard

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Objectives: Castration is a common practice in feedlot management to reduce the effects of testosterone. The two most common castration methods are surgical castration and banding. This study was conducted

to evaluate the effects of different castration methods between the treatment groups; steers (control), banding with intra-testicular lidocaine (CB), or surgical castration with intra-testicular lidocaine (CS).

Materials and Methods: A total of 144 animals were enrolled in the study. Cattle were blocked by weight at processing. There were twelve blocks in each replicate (pen). All participating animals were enrolled in the trial 40 days post-arrival to the feedyard. Individual animal data collected included: ID tag, weight at enrollment, weight at re-implant, weight 7 days pre-harvest, morbidity, mortality, and the number of cattle approaching the bunk during the first daily feeding for three days following enrollment. All statistical analyses were performed using R program. Collected data was evaluated using a randomized complete block design with a generalized linear model where replicate and treatment were specified as fixed effects and initial weight was a covariate. Contagious variables were analyzed with a linear model while morbidity, mortality and feeding behavior were evaluated in a logit model.

Results: Significant differences were noted from initial processing to re-implant for weight gain. The CB and CS groups had smaller weight gains than the control group but were not different from each other. There were no differences in weight gain between treatments from placement to harvest. A significantly higher proportion of the CB group approached the bunk than the control group. There were no differences in morbidity or mortality between treatment groups and previous morbidity did not affect growth during the study period.

Conclusions: Castration or castration method did not influence performance over the entire feeding period or behavior in the immediate period after processing. These data suggest that both castration methods when performed greater than 30 days after placement produce similar objective outcomes.

BH-019-005

Detection of bovine respiratory disease (BRD) in feedlot cattle using multiple health-monitoring systems

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Objectives: Accurate identification of sick cattle is key for effective bovine respiratory disease (BRD) control in feedlots. Unfortunately, current methods of BRD detection lack sensitivity (Se) and specificity (Sp) for detecting BRD. Monitoring systems that enable measurements of body temperature, feeding behaviour or physical activity could overcome this problem.

The objectives were to determine the Se and Sp of changes in body temperature, feeding behaviour or physical activity for BRD detection and to identify patterns that are both Se and Sp for BRD, based on simultaneous changes in body temperature and/or feeding behaviour and/or physical activity.

Materials and Methods: 561 crossbred beef steers at high risk of developing BRD (initial body-weight = 246 ± 45 kg) were observed during the first 50 days (d) after arrival at a commercial feedlot. Steers were equipped with (i) rumen temperature boluses to monitor body

temperature and (ii) three-axis accelerometers to monitor physical activity and feeding behaviour (specific system that detects the presence of accelerometers at the feedbunk). Steers with visual signs attributable to BRD (identified by pen checkers) were examined by a veterinarian. For each steer examined for BRD, 1 apparently healthy steer was selected as control and similarly examined. Serum haptoglobin (Hap) concentration was determined for each case and control. Steers with visual signs attributable to BRD, abnormal lung sounds at auscultation, and a serum Hap ≥ 0.25 g/L were defined as true positive BRD cases. Steers visually selected as controls with no abnormal lung sounds and a serum Hap < 0.25 g/L were defined as true controls. Data from health monitoring systems were retrospectively analyzed at the end of the study period using Cumulative Sum (CuSum) algorithms to detect significant behavioural and physiological changes. Se and Sp were calculated for each change alone and combined. The total number of changes detected per animal by CuSum algorithms during the 50 d study period was also calculated for each parameter alone or in combination.

Results: 34 and 26 steers were defined as true positive BRD cases and true controls, respectively. Increased body temperature was the most accurate to correctly classify true BRD cases and controls (Se = 87%; Sp = 96%); followed by decreased time at the feedbunk (Se = 71%; Sp = 71%) and decreased number of steps per d (Se = 60%; Sp = 71%). Increased body temperature was also the first to be detected with significant increases detected on average 56 h (min = 8; max = 164) prior to detection by pen checkers. During the 50 d study period, numerous changes were detected by CuSum algorithms with, on average, 4.4 (± 2.1) events of increased body temperature detected per steer, 7.7 (± 2.3) events of decreased number of steps per d and 2.3 (± 1.0) events of decreased time at the feedbunk. However, combining multiple parameters significantly decreased the number of events identified. The best combination was increased body temperature, and either decreased number of steps per d or decreased time at the feedbunk, with a Se = 83% and Sp = 96% and, on average, 2.4 (± 1.3) events detected per steer.

Conclusions: The most Se and Sp parameter to detect BRD was increased body temperature. This parameter should nevertheless be combined with other parameters such as decreased number of steps or decreased time at the feedbunk to reduce the number of detections per steer. These findings create an impetus to build new systems that simultaneously monitor multiple parameters including body temperature, number of steps per d and time spent at the feedbunk.

BW-025-001

The effect of NSAID and disbudding method on calves' behavior

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Objectives: Disbudding causes pain, but the needed length of post-operative pain medication or the possible differences between disbudding with heat cauterization and caustic paste are not known. Changes in calf activity and milk-feeder usage could be used as a measure for the calves' coping with the pain after disbudding. We studied the differences between disbudding methods and the effect of prolonged treatment with a non-steroidal anti-inflammatory drug (NSAID) on dairy calves' activity and milk-feeder visits.

Materials and Methods: We performed a 2*2 factorial design study on two-week old dairy calves weighing (mean \pm SE) 51 \pm 1 kg. We disbudded calves during sedation with either heat cauterization (CAUT n=24) or caustic paste (PASTE n=23). CAUT calves were also given local anaesthesia (lidocain). All calves got NSAID (ketoprofen) orally (4 mg/kg) before sedation (detomidine), and the NSAID5 during four more days (n=24). NSAID1 got equivalent volume of water (n=23). All calves were sham-disbudded 2 days prior (day-2) disbudding (day 0). We registered calves activity and milk-feeder visits with accelerometer and automatic-milk-feeder from day -2 to day 8. Calves were weighed days -2, 0, and 10, and calculated number of nutritive and non-nutritive visits, consumed milk, and number and duration of resting bouts and total durations for daily resting and activity. The differences between treatments were analysed with repeated sampling linear mixed models.

Results: NSAID tended to decrease daily overall activity ($p < 0.06$): NSAID5 spent less time active than NSAID1 (473 \pm 24 vs. 530 \pm 22), and NSAID5 were lying down daily longer NSAID1 calves (1022.6 \pm 18 min vs. 1002.11 \pm 18 min, $p < 0.05$). Treatment*disbudding interactions were found for the mean number and bout length of lying behavior ($p < 0.05$ for both): NSAID5+CAUT calves lie down daily more often than the NSAID5+PASTE calves did (22 \pm 1 vs. 19 \pm 1), but NSAID5 +PASTE calves had longer lying bouts than NSAID5+CAUT calves (55.43 \pm 5.0 min vs. 47.78 \pm 5.0 min). Treatment*day interactions were found for the number of all and non-nutritive feeder visits ($p \leq 0.05$ for all): NSAID5 had more visits than NSAID1 on days 3 and 4 (19 \pm 1 and 14 \pm 1 vs. 16 \pm 1 and 12 \pm 1, respectively) and non-nutritive visits on day 3 (10 \pm 1 vs. 5 \pm 1). CAUT calves drank milk portions quicker than PASTE disbudded ones (0.7 \pm 0.03 l/min vs. 0.6 \pm 0.03 l/min; $p = 0.015$). No statistically significant growth-effects were found, nor did amount of consumed milk differ

Conclusions: Disbudded calves seem to benefit from a five-day NSAID medication, as it reduced restless behaviour and increased number of feeder visits. Caustic paste calves also had a decreased drinking speed, with no effect of NSAID. We suggest that chemical and mechanical burn causes different pain after disbudding, on which NSAIDs effect differently: NSAID increased bout lengths for caustic paste disbudded calves and number of lying bouts for heat cauterized calves.

BW-025-002

A model for identifying dairies with poor welfare using a national cattle registration database

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Objectives: Consumers are increasingly demanding that the welfare of food animals is regulated. Welfare assessment protocols have been created based on scientifically valid principles. However, most on-farm welfare assessment protocols are time-consuming and expensive and there are concerns about whether they can feasibly be implemented in practice. The objective of this study was to determine whether dairy farms with a high risk of poor welfare could be identified from a national cattle database, therefore reducing the number of welfare audit visits that must be made.

Materials and Methods: The welfare of dairy cattle was assessed using the Welfare Quality (WQ) protocol on 1,930 adult animals from 24 Portuguese dairy farms. After identifying the farms with poor welfare (level 'not classified' according to the Welfare Quality protocol), 15 potential national welfare indicators were calculated based on a national cattle database (Sistema Nacional de Identificação e Registo de Bovinos, SNIRB) that contained data on births, herd movements and records at slaughter. The link between the results on the Welfare Quality evaluation and SNIRB was made using the identification code of each farm. To evaluate the probability of a farm having poor welfare, we created a decision tree using the classifier J48 of Waikato Environment for Knowledge Analysis (WEKA).

Results: Five farms were classified as having poor welfare ('not classified'), and the other 19 as having good welfare (18 'acceptable' and one 'enhanced' according to the WQ protocol). From 15 potential national welfare indicators from the national database, only two were significantly different between farms with good welfare and poor welfare. These variables were proportion of on-farm deaths ($p < 0.01$) and female/male births ratio ($p < 0.05$). The decision tree was based on the indicators proportion of on-farm deaths and proportion of calving intervals higher than 430 days to identify farms with higher risk of having poor welfare. Using this model, it was possible to correctly identify 70% and 79% of the farms classified as having poor and good welfare, respectively.

Conclusions: This study allowed for the development of a model to identify herds with poor welfare through two variables: proportion of on-farm deaths and calving-to-calving interval. Further studies should be carried out to assess the reliability of these findings for other situations. National cattle databases may simplify dairy welfare evaluation through detection of farms with a higher risk of having poor welfare. This would allow the more time-consuming welfare quality audits to be performed only on farms that are more likely to be problematic.

BW-025-003

Primary versus secondary damage in downer dairy cows

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Objectives: Downer dairy cows under commercial dairy farming conditions were monitored during their recumbency, and the importance of the primary cause of their recumbency on outcome was investigated in comparison to the effect of any secondary complications. This research was aimed at advancing the understanding of down cows, and to help improve animal welfare.

Materials and Methods: Downer dairy cows, as defined as bright and alert cows that had been recumbent for more than one day, were monitored during their recumbency under field conditions in South Gippsland, Victoria, Australia. It was an observational study of the ways farmers managed their down cows, and the 'intention to treat' was not the same for each cow. The research took place over two consecutive three-month winter seasonal calving periods in 2011 and 2012. The cause of the original recumbency of the cows was determined and allocated into one of five groups: post parturient hypocalcaemia (milk fever), protein-energy deficiency, calving paralysis, back injury or 'other'. Any secondary damage that occurred was recorded and determined if it was 'clinically important'. Status on day 7 and final outcome were recorded. For those cows that did not recover, a clinical judgement was made as to whether their demise was due solely to the primary cause of their recumbency, the secondary damage or a combination of both.

Results: There were 218 dairy cows on 96 dairy farms that fitted the study criteria. By day 7, 52 (24%) cows had recovered and 69 (32%) cows eventually recovered. The calving paralysis group was the largest group, comprised of 98 (45%) cows. When it was used as the reference group for day 3, 5, 7 and final recovery there was no significant overall effect of primary damage group ($P = 0.43, 0.44, 0.19$ and 0.41 , respectively). Some type of secondary damage was found in 183/218 (84%) cows, of which 173/218 (79%) had damage deemed to be clinically important. There were 25 different types of secondary damage recorded and 101 (46%) cows had more than one type of damage concurrently. The day 7 recovery for cows without any secondary damage was 57% (20/35 cows) compared to 14% (25/173 cows) with clinically important secondary damage (OR 7.9; 95% CI 3.6-17.4; $P < 0.0001$). Of the 149 (68%) cows that were euthanased or died, 23 (15%) were deemed to have been lost solely from the primary cause, 107 (72%) from secondary damage and 19 (13%) from a combination of both.

Conclusions: It was shown that once cows had been recumbent for more than one day there was no difference in recovery between the five primary causative groups. Clinically important secondary damage occurred very commonly and was shown to decrease the odds of recovery at day 7 by nearly eight-fold compared to cows that did not suffer any secondary damage. It was found that for the majority of the cows that did not recover, the secondary damage was more important in determining their fate than the initial cause of their recumbency. These are important findings for the welfare of downer cows.

BW-025-004

The influence of the quality of nursing care on recovery in downer dairy cows

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Objectives: Research was undertaken to investigate if the level of nursing care had an influence on the occurrence of secondary damage and on outcome in recumbent dairy cows. If so, these findings could be used to develop guidelines for 'best practice' management of down cows.

Materials and Methods: Downer dairy cows, as defined as bright and alert cows that had been recumbent for more than one day, were monitored during their recumbency under field conditions in South Gippsland, Victoria, Australia. It was an observational study of the ways farmers managed their down cows and the 'intention to treat' was not the same for each cow. The research took place over two consecutive three-month winter seasonal calving periods in 2011 and 2012. The cause of the original recumbency of the cows was determined and any secondary damage that occurred was recorded. All of the factors associated with their nursing care were recorded and a four-tiered grading system describing nursing quality, ranging from Score 1 (excellent) to Score 4 (very poor), was developed to allow analysis of its influence on outcome and on occurrence of secondary damage. The nursing grade was assigned at the completion of the recumbency on the basis of the criteria but without consideration of the outcome. Nursing Score 1 and 2 were combined to form the category 'satisfactory' nursing and Nursing Score 3 and 4 combined to form the category 'unsatisfactory nursing' for some analyses. The Cochran-Armitage test for trend was used to test for a linear component of trend between nursing scores and the proportion of cows with a given binary outcome.

Results: There were 218 dairy cows on 96 dairy farms that fitted the study criteria. By day 7, 52 (24%) had recovered and 69 (32%) eventually recovered. Some type of secondary damage was found in 183/218 (84%) cows, of which 173/218 (79%) had damage deemed to be clinically important.

There was a strong linear trend of increased day 7 recovery with increased level of overall nursing care ($P = 0.001$). There was a strong association between the occurrence of clinically important secondary damage and a decreasing level of overall nursing care ($P < 0.001$). As nursing care decreased from Score 1 to Score 4 there was a decrease in the percentage of cows recovering by day 7 from 33 to 0%, a decrease of cows eventually recovering from 45 to 0%, and an increase of cows suffering clinically important secondary damage from 68 to 100%. Some of the cows nursed 'satisfactorily' continued to recover even up to 27 days of recumbency whereas no cows nursed 'unsatisfactorily' recovered after day 5.

The level of nursing care in the study area was found to be generally sub-standard with 102/218 (47%) cows judged to be nursed 'unsatisfactorily' when first attended by the primary researcher and 67/218 (31%) nursed 'unsatisfactorily' overall.

Conclusions: The study concluded that the occurrence of secondary damage and recovery was strongly associated with the level of nursing care in downer cows. The standard of care provided by farmers in the study was often sub-standard, highlighting the need for better education of farmers, veterinarians and industry advisors in relation to nursing care. This would be expected to lead to improvements in animal welfare. The study recommends that if downer cows cannot be nursed under high levels of care, for any reason, they should be promptly euthanased to avoid unnecessary suffering as their chance of recovery is very low.

BW-025-005

A single prolonged milking interval of 24 hours compromises the well-being and health of dairy Holstein cows

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Objectives: Cows at dairy cow shows are often presented with overfilled udders in order to get a better ranking by the show judges. However, it is unclear to what extent this over-bagging during cow shows affects cows' health and well-being. Therefore, the goal of this study was to assess the effect of a single prolonged milking interval (PMI) of 24h on health and well-being of dairy cows in early- and mid-lactation and to assess the effect of a non-steroidal anti-inflammatory drug (NSAID) during a PMI of 24h.

Materials and Methods: Fifteen Holstein cows were studied in early lactation in a cross-over design and were administered NSAID and physiologic saline solution, respectively. Ten of these cows were studied again in mid lactation. Data were collected during a base line period of 24h and during a PMI of 24h in one or two hour intervals. Data of feeding and locomotion behaviour were collected using the RumiWatch®-System (consisting of a three dimensional pedometer and a noseband sensor). Udder firmness and weight distribution were measured by using a dynamometer and a four platform weighing scale, respectively. The abduction of the hind limbs was assessed while standing (indirect measurement of abduction by analysing digital images) and walking (subjective score; 0-100). Occurrence of milk leakage and oedema formation in the subcutaneous udder tissue was assessed by visual observation and ultrasound examination, respectively. Data of the last 6h of a 12h milking interval were compared with the last 6h of the PMI.

Results: The behaviour of cows in early lactation (without NSAID) was significantly changed during the last 6h of PMI, as we observed decreased eating time, increased ruminating time and hind limb abduction while walking and standing. Udder firmness was increased during this period and relatively more weight was placed on the hind limbs. Pathological signs were found at the end of PMI: 100% of cows showed milk leaking and two thirds of cows developed oedema in the subcutaneous udder tissue.

Administration of NSAID did not influence variables substantially, except for oedema formation, which was not significantly increased during PMI in the NSAID-group. In the cows in mid-lactation, different variables were no longer significantly changed (e.g. eating and ruminating time, occurrence of oedema and the abduction during walking).

Conclusions: It is concluded that cows' health and well-being are compromised by a single PMI of 24h, as the behaviour is changed (decreased eating time, increased ruminating time and hind limb abduction) and different pathological signs were recorded (oedema in subcutaneous udder tissue and increased milk leakage). NSAID had only a slight effect on the well-being of cows during a PMI. Otherwise, a single PMI has less effect on the well-being of cows in mid-lactation, as fewer variables were changed significantly as compared to cows in early lactation.

BW-025-006

Practices for the disbudding and dehorning of dairy calves by veterinarians and dairy producers in Ontario, Canada

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Objectives: The objective of this study was to build on a 2004 survey of bovine veterinarians and dairy producers in Ontario (Misch et al., 2007), to determine if disbudding and dehorning (DD) practices had changed over the past decade, and to examine factors associated with the adoption of pain-control.

Materials and Methods: An online survey (Qualtrics, 2005. Provo, UT), with an option to participate via telephone, was conducted in the fall of 2014. Members of the Ontario Association of Bovine Practitioners (n=238), and a subset of Ontario dairy producers (n=603, stratified by 15 geographic zones) were invited to participate. Both surveys included the following sections: demographics, DD practices including use of pain control, perceptions of pain, and knowledge of the Canadian Code of Practice for the Care and Handling of Dairy Cattle. The producer survey also included questions on use of polled genetics, and general calf management.

Perceptions of pain were evaluated by use of an ordinal Likert scale, with 0 being no pain, and 10 being the worst pain imaginable. Both producers and veterinarians were given the same eight procedures/conditions cattle may experience, and asked how painful they thought the animal would be, assuming no anesthetics or analgesics were given unless stated otherwise. The eight procedures/conditions were: dystocia (hard pull), severe mastitis, sole ulcer, right flank surgical correction of left displaced abomasum (with a local block given), ear tagging at birth, caustic paste applied at two days of age, small diameter thermal disbudding at two weeks of age, and surgical amputation and cautery dehorning at five months of age.

Results: Ninety-three veterinarians (39%) from 51 clinics (63%) and 165 dairy producers (27%) completed the survey.

Ninety-four percent of responding clinics reported veterinarians or veterinary technicians perform DD for a mean of 29% (SD=20) of their dairy clients. Compared to the 2004 veterinary survey, significant increases were seen in the use of local anesthetic (99% vs. 92%, p=0.013) and NSAIDs (59% vs. 1.5%, p<0.001). Injectable meloxicam represented 89% of all NSAIDs used.

Dairy producer respondents had a mean age of 44 years old (SD=13, range=16-74). Farms had from 20-450 milking cows (mean=82, SD=71). Seventy-three percent of farms performed DD themselves. Of these, 62% used local anesthetic, 38% used sedation, and 24% used an NSAID. The use of all three medication types was significantly greater than 2004. Sixty-three percent of producers reported changing DD practices in the past 10 years. Common changes included addition of local anesthetic (60%) and performing DD at a younger age (30%). The most common influence cited for changes was the herd veterinarian (73%).

Factors associated with use of local anesthetic by producers were assessed in a logistic regression model. Positive associations included: herd health visits every two weeks or sooner (OR=3.9, p=0.018), and increasing % of calves DD at >8 weeks of age (OR=1.01, p=0.045).

Differences in likert scores between veterinarians and producers were evaluated for all 8 procedures/conditions. There were no differences in median score (Hodge Lehman median difference test) or equality of distribution (Kolmogorov-Sminrov test).

Conclusions: The use of local anesthetic in this survey was far higher than previously reported. NSAID use by veterinarians was also far higher than previously reported. Within the survey populations, producers and veterinarians appeared to view the experience of pain by cattle similarly. A strong relationship between producer and veterinarian was associated with the adoption of pain-control. Veterinarians can clearly play a key role in improving DD practices for dairy calves. Identifying factors associated with adoption of best practices may help veterinarians target appropriate educational opportunities for their dairy clients.

BV-033-001

From control to surveillance – the Swiss Bovine Viral Diarrhoea (BVD) eradication programme

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Objectives: We inform about the experiences from the Swiss Bovine viral diarrhoea (BVD) eradication programme. In the programme the shift from control to surveillance continued in the last few years. We show the relative importance of case measurements and measurements on farms known to have a higher risk for BVD persistence and re-infections compared to large scale testing. We discuss possibilities to keep the time period with low frequency cases (epidemic tail), following the main clearance, short.

Materials and Methods: In animal disease eradication programmes, determining the time point to shift from control to surveillance is of crucial importance for the effectiveness and the economy of the programme. As control comprises usually large scale testing, it should be as short as possible. Therefore, the shift to surveillance should take place as early as possible. On the other hand, shifting too early to surveillance increases the risk of leaving new infections unrecognized. Traditionally, the shift is done following an extended time of control testing without revealing any cases. In BVD programmes time intervals are long. Thus, there is often a need to change to surveillance even in the presence of new infections.

The Swiss BVD control programme was based on the detection and elimination of persistently infected animals. This was done by testing for antigen or by PCR. After virological testing of all cattle, all newborn calves have been tested for BVD virus for 4 years until the end of 2012. In 2012, serological tests twice a year of all dairy herds and of a third of the remaining farms was done additionally to calf testing. In 2016 the testing programme will be reduced to once per year for dairy herds whereas it will not change for other herds. However, as case numbers are low, but do not decrease further, measures to control case herds more efficiently are applied since autumn 2015.

We analyse the data from the BVD control programme descriptively, showing the rationale for lowering surveillance while increasing case control measures. We present examples for infection chains during control.

Results: In October 2015 only 30 herds out of a population of 40,089 cattle herds are closed due to BVD. About 99.9% of the herds are free from BVD. Only about 80 herds still have single cows blocked because of known or likely exposure to BVD cases during the susceptible pregnancy time. The prevalence of persistently infected animals (PI) in newborn calves has decreased from 1.4% in 2008 to 0.03% in autumn 2015. In spring 2015 96% of the dairy herds showed no or only few antibodies in bulk milk samples. Due to the surveillance scheme, the figures for non-dairy herds cannot be derived easily, but indicate the same trend. Varying causes were responsible for the prolonged persistence of BVD on a small scale. The causes depended on the testing scheme and the eradication phase. First, during the calf testing, some of the herds could not be cleared from BVD. This was caused by poor herd management and faults in sample taking. Second, when changing from calf testing to serological testing, bovines tested false negative for BVD virus caused some damage, although the absolute number of such animals was small. Third, in the phase of serological testing, problems arising from data inconsistencies in the animal movement database hindering effective tracing forward and backward and ambiguous herd identifying are the main problems. In 2015 we observed about 30 re-infections of herds previously free from BVD. Interestingly, about 95% of the cattle herds have been free from BVD throughout the whole programme.

Herds with a history of BVD were at higher risk to get re-infected.

Conclusions: For BVD eradication, it is of high importance to consider the weaknesses and strengths of the testing scheme and the eradication phase. During calf testing, prevention of within herd transmission is of major importance as is the true sensitivity of the whole sampling and testing chain. During serological testing, reliable data of animal movements and herd connections are of higher importance, as the time interval from infection to detection of affected herds is longer. In contrast, determining a BVD negative status of a herd from calf testing is not possible. Virustyping is of importance to investigate infection chains between herds.

BV-033-002

The Scottish BVDV Eradication Scheme: Are we making progress?

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Objectives: Over the past 20 years, BVDV control and eradication programmes have been used throughout Scotland, predominantly in the beef sector. Following extensive consultation, the Scottish Government considered that a national eradication scheme would benefit the cattle industry. The motives for eradicating BVDV included: improving the financial sustainability of cattle production; improving the welfare of cattle; and reducing greenhouse gas emissions per kilogramme of milk and beef produced by increasing the efficiency of production.

Materials and Methods: The Scottish BVDV eradication scheme has taken a phased approach. In phase 1, which ran from September 2010 to April 2011, the Scottish Government subsidised testing in order to encourage farmers to determine their BVDV status. Legislation was put in place for phase 2. This required all breeding herds to screen for BVDV by February 2013 and annually thereafter. Farmers and their veterinary surgeons could choose from one of six different testing methods: the antibody check test (spot test); calf screen for virus; whole herd screen for virus; or one of three options using bulk milk sampling for antibody. Further legislation was put in place for phase 3 in January 2014: the herd status had to be declared at the point of sale; movement restrictions were put on untested herds; and animals persistently infected with BVDV could only be moved directly to slaughter. Phase 4 commenced in June 2015. The number of screening options were reduced by the removal of the bulk milk testing methods. Movement restrictions were put on herds with a "not-negative" status and there are now testing requirements for animals entering herds from untested holdings.

Results: A survey carried out prior to the eradication scheme indicated that 40% of Scottish breeding herds had evidence of exposure to BVDV. The percentage of beef herds classified as "not-negative" has fallen from 23% in 2012 to 9% in September 2015. The percentage of dairy herds with this status has fallen from 52% to 31% in the same time period. Currently 12% of breeding herds in Scotland are classed as "not-negative". To date 3,128 animals have been identified as infected with BVDV; of these 514 are still alive on holdings in Scotland. BVDV results from all approved testing laboratories are held in a central database that is used by farmers, markets, veterinary surgeons and government.

Conclusions: The Scottish BVDV eradication scheme has made considerable progress over the past five years. The phased approach has ensured that all sectors of the cattle industry are aware of their increasing responsibilities. This progress is comparable with

Scandinavian countries that have taken a similar approach to eradication. In these countries this began with voluntary schemes and progressed to compulsory testing with eradication typically achieved in ten years.

BV-033-003

Results of the first year of the mandatory BVDV control program in Northern-Belgium

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Objectives: During decades, the control of bovine viral diarrhoea virus (BVDV) in Belgium was on a voluntary basis. Initiatives of eradication at the herd level were successful if well-performed, but the absence of a systematic form of control of BVDV resulted in a high risk of (re)-entry of the virus in all cattle herds in Belgium. Starting from the first of January 2015, a national BVD-program was initiated for Belgium. The main goal of the program 'STOP BVD', is to eradicate the virus. This abstract discusses the layout of the Belgium BVDV control program and gives the results of the first year of the program.

Materials and Methods: The pillar of the eradication program is to detect persistently (PI) infected animals in an early phase after birth. Similar to other European countries (e.g. Germany and Ireland), all newborn animals have to be tested for BVD-antigen, this within 7 days after birth. This systematic 'newborn testing' preferably happens through an ear notch sample taken by the farmer.

The results presented in this abstract are based on the national BVD-database which was installed and coupled with the I&R-database. Different states included in the program are: PI-free, PI-free by descendance, PI, and PI-suspect and BVD-state unknown. PI and PI-suspect animals are prohibited from trade, and PI-animals are encouraged to be culled. Animal BVD-states are listed on the animals ID and available for all farmers. In case of the detection of a PI, veterinarian herd visits to sample suspect animals and are encouraged to trace other PI animals at the farm, coupled with other measures to control BVD.

Results: The results presented in this abstract only include the first 11 months of the program. During this period, 440.447 newborn animals were tested for BVD-antigen using ear notch samples. 2.690 samples were empty (i.e. ear notch sampling unsuccessful) in which a new sample was taken by the herd veterinarian. 2.556 newborn calves tested positive and were classified as PI animals on 1.179 cattle herds in Northern-Belgium. Although in this stage of the program, culling of PI-animals remains voluntary, on average 90% of PI animals of newborn calves were euthanized. Through testing of the dams of this PI-animals, again 187 PI-dams were detected through sampling of the herd-veterinarian (i.e. about 10% of mothers of a PI-calf are itself a PI-animal). During the first year, another 359 PI animals were detected between 3 months and 2 years of age and another 381 detected PI animals were older than 2 years. The systematic testing of newborn animals coupled by the certification by descendance resulted that 957.045 cattle in Northern Belgium are certified as PI-free.

Conclusions: The results of the first year of the 'STOP BVD' program illustrates Belgium was in need for a systematic approach of BVDV-control. Approximately 6 out of 1.000 newborn calves were PI animals. PI-animals were detected 1.383 cattle herd or about 9.2% of cattle herds in Northern-Belgium. Through systematic testing of newborn animals, about 78% of cattle were certified PI-free for live.

BV-033-004

Application of epidemiological and economic modelling to the Irish BVD eradication programme

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Objectives: Bovine Viral Diarrhoea (BVD) is an infectious disease of economic importance across the world. The production losses in BVD-affected farms have motivated many national control programmes. In 2013 Animal Health Ireland (AHI) initiated the compulsory phase of the Irish national BVD eradication programme based on ear notch antigen testing of newly born calves. The objective of our study was to conduct a comparative epidemiological and economic assessment to understand the benefits and pitfalls of introducing herd level serological testing (Option 2) as an alternative surveillance strategy to individual animal virus testing (Option 1).

Materials and Methods: The comparative evaluation of surveillance strategies was performed with a spatially explicit herd-level simulation model. The model was tailored to represent the farm and national dimension of the Irish cattle sector. It comprises approximately 100,000 individual cattle farms, their location, size, management groups and breeding schedules, contiguity on land parcel level and the complete record of cattle movements between Irish farms replicated by source, target and age data. Farm behaviour related to the retention of PI animals was also included. Secondary patterns reported from AHI programme data were used to calibrate the model. The model was run for 5 years of BVD endemicity (i.e. corresponding to 2008-2012) followed by 10 years of control programme (i.e. 2013 onwards). The simulated measures utilised Option 1 for 3 years, continuing (i.e. from 2016) with either Option 1 or 2. The latter including a temporary return to virus testing on farms with a BVD positive diagnosis. Key model outputs included the number of farms with BVD, the number of calves detected as PI per year, the quantity of testing applied under the various control programmes, and assessment of pathways leading to breakdown of BVD negative farms e.g. 'Trojan' cow movement or across-the-fence transmission.

Results: If retention of detected PI calves was excluded, both surveillance options resulted in BVD eradication, with only a slight delay associated with Option 2. Costs of diagnostic testing differed at the national level and were lower for option 1 because a BVD breakdown in a herd using serological screening would require a return to tag testing for some years. The risk of farm breakdown declined as the programme progressed. There was a tipping point in cost ratio, depending on farm level prevalence, beyond which the expected future costs would favour Option 2.

However, when the level of PI retention, as reported from AHI programme data, was modelled, the simulation outcomes were less optimistic. Eradication was delayed with a doubling of the control time, or was not achieved within the 10 year time window of the simulation.

Conclusions: The retention of PI animals by some farmers is hindering the effectiveness of the simulated BVD control programme by lengthening time to eradication, increasing the cost of eradication and jeopardising control efforts on BVD-negative farms. In the absence of PI retention, in time it would become cost-effective to change from individual calf virus testing to a farm-level serological surveillance approach.

BV-033-005

Economic evaluation of bovine viral diarrhoea virus control activities worldwide: a systematic review

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Objectives: The primary aim of this study was to assess studies with a specific emphasis on the costs and benefits of control activities of the bovine viral diarrhoea virus (BVDV) at national, regional and farm level. The second aim was to collect data with respect to direct (production) losses and indirect losses caused by the costs of BVDV control, as well as to the net benefits and costs of these control activities. Furthermore, we hypothesized that countries for which estimates of production losses are recorded also carry out economic assessments of their control activities.

Materials and Methods: An extensive literature search covering research from 1970 to 2014 was performed between December 2014 and July 2015 using the online databases PubMed, ISI Web of Science and Scopus. The search term "BVDV" was combined with the keywords "control program/me", eradication, intervention, mitigation, surveillance, freedom of disease, biosecurity, Scandinavian strategy, "disease losses", cost analysis, cost benefit, economics, economic models, expenses and financial impact.

Each paper was first reviewed in full and the economic content (if available) was evaluated based on predefined economic assessment criteria ranked between high quality (1) and low quality (4). High quality studies include an economic analysis of control activities, the allocation of the costs and benefits per player as well as quantitative values of the economic assessment of control activities. The low quality studies do not include an economic analysis in their methods, but describe the economic outcome of their studies in passing and provide only qualitative values for control activities. All economically relevant studies (primary studies) were reviewed according to their location, assessment level (national, regional, industry, farm), aims, methodology, production types, metrics, outcomes and research field (e.g. epidemiology and risk; diagnostic and genomic analysis; vaccines and vaccination strategy). In the next step, the references of the primary studies were screened by title and abstract. Of these, all articles that were considered relevant were also reviewed in full (secondary studies).

Results: Overall, 29% (n=144) of the identified articles (N=501) included information on economic assessments of BVDV control activities. Of these 144 articles, approximately 18% could be allocated to national, 25% to regional, 27% to farm level and 30% to no explicit geographical category. The most frequent aim of these studies (33%) was to describe or evaluate the eradication/control programmes or prevention approaches, followed by the description, evaluation or development of diagnostic methods or approaches (20%). Studies with the highest quality levels of the economic assessments were found in the United Kingdom, Norway and France, and studies with good quality levels were identified in the United States, Switzerland, New Zealand, Netherlands, Ireland, Denmark and Canada.

In total, 50% of the studies made direct reference to cattle in general, with an additional 21% analysing dairy farms, 6% beef farms and 4% calves and youngstock. In the remaining 19%, no mention of bovine species or production type was made. Furthermore, 16% (n=80) of the studies provided data on direct losses due to reduced production.

The studies with high quality assessments were found in 23% and the majority studies were conducted on the farm level.

Conclusions: Our systematic review shows that most studies (69%; [99/144]) on BVDV provided only qualitative values of control activities and did not include an economic methodology in their study design. This lack of economic assessment is also visible in the calculation of the production losses. Our hypothesis that countries which record production losses are also likely to carry out economic assessments of control activities could not be confirmed in full. Some countries calculated production losses but did not provide an economic assessment of control activities or did not conduct systematic control activities against BVDV.

BV-033-006

The importance of the number of animals tested and threshold at which a herd is deemed positive for BVD when testing young-stock for BVDV antibodies.

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Objectives: The objective is to demonstrate some examples of why vets in practice should take into account the number of animals tested and the threshold of positives at which the veterinarian or, if it is part of a scheme, then the scheme decides to designate that herd as positive. It is likely that to some extent veterinarians do this intuitively but it is also useful to have quantitative estimates of this for reference.

Materials and Methods: Prior to the start of the BVD eradication scheme in Scotland, a random survey of Scottish beef herds was undertaken and blood samples from ten young stock per herd were tested for BVDV antibody. Using the results from this survey it was possible to randomly sample from the results to see how many of the herds would have tested the same way depending upon (a) the number of animals tested and (b) the threshold of positive animals at which a herd is deemed positive.

Therefore we are able to present evidence of the effective herd-level sensitivity and specificity across different combinations of the number of animals tested and thresholds chosen.

Many schemes aiming to eradicate BVD choose, as a pillar of the screening system, the testing of blood of ten young-stock animals for antibodies to BVDV. If one or more animals tests positive then the herd is deemed positive. In some herds, the number of young-stock animals available for this screening process is fewer than ten. This study makes use of data from a random survey of beef farms in Scotland, prior to the Scottish eradication scheme, to show that the number of animals tested and the threshold of positive animals at which a herd is declared positive has a hugely important effect on the overall herd level sensitivity and specificity of this method. Veterinarians should be aware of this when interpreting their results.

Results: The herd sensitivity (i.e. the probability of designating the herd as positive given that it is truly positive) is greatly dependent on both the number of animals tested and the threshold at which we call the herd as positive. Unsurprisingly the herd sensitivity goes up with the former and down with the latter and we employ graphs to demonstrate this pattern based on our specific estimates of those different scenarios.

The herd specificity (i.e. the probability of designating the herd as negative given that it is truly negative) behaves in a fairly similar but reverse manner and we employ graphs to demonstrate this.

Conclusions: Whilst many veterinarians and farmers, probably intuitively, account for both the number of animals tested and the number of animals testing positive for BVDV antibody when interpreting their results, it is possible to give some empirical evidence in order to estimate how they would do this in a more quantitative way and this may be of interest to vets, farmers or those planning eradication schemes.

eliminate interference of colostrum-derived specific antibodies with BVDV antigen detection.

Comments: This work has been accepted for publication by the Journal of Veterinary Diagnostic Investigation.

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BV-033-007

Heat treatment of serum from calves PI with BVDV to remove interference of colostrum-derived specific antibodies with antigen detection by ELISA

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Objectives: Bovine viral diarrhoea (BVD) is one of the most prevalent and economically significant viral diseases of cattle worldwide. A range of diagnostic tools are available for the detection of both viral antigen. The antigen ELISA is a rapid, inexpensive and robust test that successfully identifies persistently infected (PI) cattle. However, the antigen ELISA is subject to interference from colostrum-derived specific antibodies in serum of neonatal and young PI calves. This study investigated methods to remove specific antibodies from serum samples and, therefore, reduce interference.

Materials and Methods: Serum samples containing BVDV antigen and/or specific antibodies were treated by methods adapted from More and Copeman (Trop Med Parasitol 1991;42:91-94): treatment with heat (boiling) with or without the addition of an equal volume 0.1M EDTA pH 4.5, 5.5, 6.5 or 7.5. Treated samples were centrifuged and the supernatant recovered. All treated and untreated samples were tested for detectable BVD viral antigen and specific antibodies by ELISA (IDEXX BVDV Serum/Ag Plus ELISA and IDEXX BVDV Total Ab ELISA, respectively). The optimal treatment was identified and applied to serum samples collected longitudinally from three PI and seven non-PI calves from the day of birth to five weeks of age. As above, all treated and untreated samples were tested for detectable BVD viral antigen and specific antibodies by ELISA.

Results: All treatments resulted in an increase in antigen signal and a decrease in detectable antibody levels. Optimal treatment was identified as heat treatment with the addition of an equal volume of 0.1M EDTA pH 5 ± 0.5, resulting in antigen signal recovery in excess of 90%. When applied to field samples collected longitudinally from three PI calves from birth until five weeks of age, all treated samples tested positive for antigen at all time points. By comparison, the same samples when untreated returned negative antigen results for between six days and four weeks post-colostrum ingestion. The vast majority (98.6%) of samples from non-PI calves collected after the day of birth tested negative for BVDV antigen. On the day of birth, four of seven non-PI calves returned (low) positive results.

Conclusions: Heat and EDTA treatment of serum samples presents an opportunity to improve performance of BVDV antigen ELISAs for testing of colostrum fed calves. Diagnostic sensitivity was improved, however, diagnostic specificity was decreased substantially on the day of birth. The data indicates that adjustment of the positivity threshold is appropriate and will result in excellent diagnostic performance. Further study on larger numbers of calves is warranted, but the results of this study indicate that treatment of serum samples has the potential to

CH-044-001

Them And Us: Understanding How Farm Consultants View The Veterinary Profession

A Survey Of 25 Farm Consultants In New Zealand

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Objectives: In New Zealand, farmers often rely on the input of private agricultural consultants in transferring technical and scientific research findings into practical changes in farm management. These activities will involve interactions with other rural professionals including veterinarians. This study was designed to assess the attitude of dairy farm consultants to the role of vets on dairy farms and whether they viewed veterinary advice as impartial, free from commercial bias and central to farm productivity and profitability.

Materials and Methods: A survey was commissioned in 2014 by Vetlife (Vetlife Administration Support Office, 82, Sophia Street, Timaru) – a mixed veterinary business in the South Island of New Zealand – using Cinta Research Ltd (Havelock North, Hawke's Bay, New Zealand) - a quantitative and qualitative research service specialising in the New Zealand agricultural industry. A formal telephone survey was conducted with dairy farm consultants in the Canterbury and Otago regions delivering a full time, fee earning business advising farmers to explore their attitudes towards the veterinary profession.

As an introduction, descriptive data was collected on the business model of the consultant's practice and experience. The first part of the questionnaire contained questions designed to elicit either numerical answers which could be categorised as a continuous variable or used a Likert scale from one to five measuring agreement with a statement and yielding ordinal variables. The second part consisted of six questions, the unprompted answers to which were collated and matched to a series of non-disclosed categories for each question. The last three questions were designed to allow the capture of more general pre-prepared statements. Statistical analysis was carried out where appropriate to assess numerical answers and agreement with statements.

A pilot survey was carried out with a full time rural farm consultant outside the group selected for participation to ensure that questions were clear and unambiguous.

Results: Just over half (56%) of the respondents worked as sole practitioners and 43% as part of a larger group. The median working experience of the group was 3-5 years. The answers that were collated from 25 respondents indicated that whilst consultants respect veterinary advice they see themselves as the custodians of animal health expenditure on farms. This is seen as an expense to be controlled with successful animal health characterised by low expenditure. This view was held more strongly by consultants with more than five years of professional experience. Interventions by vets are seen primarily in terms of treatment of sick animals, provision of animal health products and not as key drivers of farm profitability. Consultants feel they engage widely with farmers with major roles in the influence of animal health on farm productivity, nutrition and reproduction. They believe that animal health is a driver of productivity but they do not believe that veterinary led expenditure on animal health will deliver increased profitability.

Conclusions: Consultants are ambivalent about the value of working more closely with vets and about the benefit that closer veterinary intervention brings to the farm's profitability. For farm vets, better identification of farm goals, more team work with other rural professionals

and a wider understanding of the impact of veterinary actions at the farm level may help them to break down these barriers. Better understanding of how other rural professionals involved in a farm's management view vets will help to develop more successful veterinary involvement on farms, characterised by a deep understanding of the individual farmer's goals and motivations.

CH-044-002

Comparative losses due to liver fluke (*Fasciola hepatica*) in key sectors of the British cattle industry.

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Objectives: Liver fluke is a common, ubiquitous parasite that affects the health and welfare of cattle worldwide. The costs incurred, via production losses and liver condemnations, and their effects on the economics of the British cattle industry are not entirely clear. In order to effect change and reduce reliance on drugs, we must demonstrate that any improved means to control the disease and reduce infection cost less than the benefits accrued from their application. Our objective was to develop bio-economic models that provide quantitative estimates of the losses incurred in alternative cattle production systems, as a first step in this process.

Materials and Methods: Herd-level stochastic models for growing animal, beef suckler herd and dairy enterprises were developed and implemented using @Risk. Each of these models can be modified to simulate the major management systems used (e.g. spring/summer calving suckler herd vs autumn/winter calving suckler herd) and, in the case of the dairy model, to account for a range of average 305-day milk yield per cow of between 5500 and 9000 litres. Linked to partial-budget models, they have been run for fluke vs. no fluke. Variables were parameterised with estimates derived from a variety of sources including: literature, publically available data, industry and expert opinion. Sensitivity analysis was performed to identify the most influential parameters in the models

Results: Spring/summer calving suckler herds have higher losses per infected cow than those calving in autumn/winter. In the growing animal model, spring/summer calving born beef replacement heifers herds have higher losses per infected animal than those born in autumn/winter; however the average loss per infected animal (£/year) is lower for spring/summer born beef finishers than others. Higher losses are experienced for growing heifers originating from suckler herds than from dairy herds. Dairy herd losses increase with increased milk yield; the median overall loss per infected cow is £186/year (range minimum in low yield herds £149-261 maximum in high yield herds). The main drivers differ between the models but reduction in performance parameters (e.g. milk production in the dairy model, average daily live weight gain in the growing animal model) and prevalence of fluke infection feature commonly.

Conclusions: We concluded that, at herd-level, liver fluke has a significant financial effect and we need better estimates of the effect that control methods have on the prevalence of infected animals. Outputs from these models will be aggregated to estimate national/regional net benefits of improved control of fluke of use for policy decision support. In later stages of the work, the suite of models will also provide a means to rank (by cost-benefit) potential control measures based on improved management practices.

CH-044-003

The costs of dairy production disorders in European practise; over- or underestimated?

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Objectives: For veterinarians and animal health advisors to give a good advice to the dairy farmer it is vital that the costs of disease are estimated correctly. Failure to do so may lead to wrong priorities in animal health management advice. The first objective of our study was thus to explore the costs of mastitis, lameness, ketosis and metritis as estimated in practise by farmers, veterinarians and animal health advisors. The second objective was to estimate the costs of each disease using a disease costs estimation tool. The third objective was to study whether farmers, veterinarians or advisors tended to over- or underestimate the costs of disease.

Materials and Methods: A total of 60 German, 21 Spanish and 40 Swedish organic dairy farmers were asked to participate in this study and to make their herd health records available for the cost estimations. The veterinarian and farm health advisor of each participating farm were asked to participate along with the farmer to calculate the farm specific cost. This study was part of a larger study on the improvement of animal health on organic dairy farms in Europe (IMPRO, 7th Framework).

To estimate the disease costs of mastitis, ketosis, lameness and metritis a cost estimation tool was constructed. In which disease costs comprised the following parts; milk production losses, discarded milk, treatment, culling and destruction. Technical model input included: number dairy cows, number of cases of clinical mastitis, SCC, fat/protein ratio, treated cases of ketosis, number of culled or death cows. Economic model input included: milk price, feed costs, costs of labour, slaughter value, replacement value, penalties paid and bonuses missed. For each economic input value a country specific reference value was given.

During each farm visit farmers, veterinarians and advisors were first presented with the overall performance of the dairy herd (e.g. milk yield, number of cows) and current health situation (e.g. number of (sub)clinical cases, SCC and lameness scores) and asked to estimate the total costs for each disease separately and solely based on these records. Second the costs estimation tool was performed and results were presented and discussed with the farmer, veterinarian and advisor.

Results: A total of 60 German, 21 Spanish and 40 Swedish farm visits were performed. In which, respectively 35, 28 and 40 German, 13, 15 and 7 Spanish and 27, 16 and 21 Swedish dairy farmers, veterinarians and advisors participated. The remaining participants did not estimate the costs of disease since they felt not qualified to estimate these costs.

The costs of mastitis on herd level were assessed by the disease costs estimation tool, at an average of €8,050 /yr., €10,613 /yr. and €11,099 /yr. for German, Spanish and Swedish farms. The costs of lameness were assessed at an average of €4,579 /yr., €2,565 /yr. and €3,357 /yr. for German, Spanish and Swedish farms. The costs of ketosis were estimated at an average of €2,152 /yr., €345 /yr. and €2,405 /yr. for German, Spanish and Swedish farms, respectively. The costs of metritis were estimated at an average of €2,060 /yr., €477 /yr. and €460 /yr. for German, Spanish and Swedish farms, respectively.

Over- and underestimating the costs of each disease by more than 50% of the assessed costs was found to be a common practice among farmers, veterinarians and advisors. The costs of mastitis, lameness, ketosis and metritis were on average underestimated by more than 50% in respectively 32%, 40%, 49% and 40% of all cases and overestimated

by more than 50% in respectively 19%, 23%, 27% and 45% of all cases. During a farm visit, farmers, veterinarians and advisors gave estimates which were, generally, in-line with each other's response.

Conclusions: Disease costs varied substantially within a country due to different input, either economic or technical. The farmers, veterinarians and advisors were found to either overestimate or underestimate the costs of production disorders by a substantial amount. Besides, some farmers, veterinarians and advisors found themselves unqualified to estimate the costs. Because economic arguments are, at least partly, a reason to adopt preventive measures or not, these results stress the importance to use a structured framework when estimating the costs of production disease on-farm, before advising preventive measures.

CH-044-004

Expert opinion disability weights for the Dairy Disease-Adjusted Lifetime (DAL) metric

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Objectives: Over the past 175 years, data related to human disease and death have progressed to a summary measure of population health, the Disability-Adjusted Life Year (DALY). As dairies have intensified there has been no equivalent measure of the impact of disease on the productive life of animals. The development of a disease-adjusted metric requires a consistent set of disability weights that reflect the relative severity of important diseases. The objective of this study was to elicit dairy disability weights to be used in an assessment of the burden of disease on animal well-being and consequent opportunity costs of reduced productive life.

Materials and Methods: National and international dairy health and management experts were contacted through professional organizations, dairy industry publications and conferences, industry contacts, and word-of-mouth. Estimates of minimum, maximum, and most likely disability weights were established for calving trauma, diarrhoea, ketosis, lame (hoof only), left displaced abomasum, mastitis, milk fever, metritis, musculoskeletal injury (leg, hip, back), pneumonia, retained placenta, and right displaced abomasum. Survey participants were asked to rank the impact of each disease on overall health and milk production. Diseases were classified from 0 (no adverse effects) to 10 (death). The respective weights were dependent upon the estimated duration of clinical and subclinical disease inclusive of periods of treatment and recovery. The expert data was modelled using modified BetaPERT distributions to demonstrate the variation in these dynamic disease processes, and to identify the most likely aggregated disability weights for each disease classification. The PERT distribution was specified for each set of expert opinions (minimum, most likely, maximum), using the following formula: $PERT(a,b,c) = Beta(\alpha_1, \alpha_2) * (c-a) + a$. The individual distributions were combined using a discrete distribution in the form of $Discrete(\{x_i\}, \{p_i\})$, where $\{x_i\}$ were the expert opinions for experts $i = 1$ to n and $\{p_i\}$ were the equal weights given to each expert opinion.

Results: A total of 96 experts responded with estimates of disability weights. Veterinarians comprised the majority of respondents (83%), and most respondents were within North America (89%). The results indicated the following ranking of disease from least to most severe using

an average of the medians with the minimum, most likely, and maximum medians in parentheses: retained placenta (2, 3, 6), diarrhea (1, 3, 8), ketosis (2, 4, 8), metritis (2, 4, 8), mastitis (2, 4, 10), milk fever (2, 4, 10), lame (2, 5, 9), calving trauma (2, 5, 10), left displaced abomasum (4, 5, 9), pneumonia (3, 5, 10), musculoskeletal injury (3, 6, 10), and right displaced abomasum (4, 7, 10). The peaks of the probability density functions indicated that for certain disease states there was a relatively narrow range of expected impact whereas other diseases elicited a wider breadth of impact. For example, although mastitis and milk fever had equivalent overall distributions, the experts' average distribution width was narrower for milk fever than for mastitis. This suggests that for certain diseases such as milk fever, experts were confident about their estimate with the minimum, most likely and maximum scores closely aligned per respondent. For other diseases, it is likely that a more nuanced definition of disease with additional unique health states may be required to expand estimated impacts. This was particularly apparent with respect to mastitis, lameness, calving trauma, and musculoskeletal injury, all of which could be redefined using gradients of severity or accounting for sequelae.

Conclusions: These disability weight distributions serve as an initial step in the development of the DAL metric. They will be used to assess the time lost due to dynamic phases of dairy cow diseases and injuries. Prioritizing health interventions based on time expands the discussion of animal health to view profits and losses in light of the quality and length of life. The dairy industry needs such a measure to move beyond simply monetizing health issues, i.e. the cost per case, to a more thorough assessment of the implications of time lost due to illness or injury, forced removal, or death.

CH-044-005

Direct economic cost of bovine respiratory disease in US beef calves prior to weaning

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Objectives: Bovine respiratory disease (BRD) is a leading cause of sickness and death in pre-weaned beef calves. The economic loss due to BRD in feedlot cattle has been reported, but similar information for beef calves prior to weaning is not available. The objective was to estimate the direct economic cost of BRD in beef calves prior to weaning.

Materials and Methods: Data were obtained from USDA reports (NAHMS 2007-2008, NASS 2014, and AMS 2014), peer-reviewed papers, Health records (9,921 calves from 28 cattle management groups within 7 Nebraska, USA beef cattle ranches with BRD), and internet based pharmacies. A stochastic simulation model was conducted using computer spreadsheet add-on software. The model was comprised of three parts, cost of BRD mortality, cost to treat BRD, and losses associated with decreased weaning weight from BRD based on the beef cow inventory in 2014. The input variables included percentage of beef cows that calved, percentage of calves born alive, percentage of calves born alive but died before weaning, percentage of deaths at any pre-weaning age due to BRD, weaning weight of calves, 2014 market price of calves weighing, BRD incidence rate, weight lost due to BRD, and cost to treat a calf for BRD, including estimates for antibiotic costs. Cost for labor to treat sick calves and the costs of diagnostic testing were not included.

Results: The results showed the estimate of the total direct economic

cost of BRD in pre-weaned beef calves for 2014 was \$200.3 million (90% CrI 167.6-236.4), of which the total death losses, medical treatment cost and weight loss cost were \$167.0 million (90% CrI 135.4-201.9), \$10.8 million (90% CrI 3.6-17.8), and \$22.5 million (90% CrI 15.9-30.0), respectively. Costs associated with death due to BRD in calves <3 weeks and ≥3 weeks of age were \$56.7 million (90% CrI 41.0-75.3) and \$110.3 million (90% CrI 82.7-142.0), respectively.

Conclusions: We estimate that BRD in pre-weaned beef calves most likely cost US cattle farmers \$200.3 million in 2014. Of this, the largest component (55%) was death in calves ≥3 weeks of age. Therefore, to most effectively minimize the cost of BRD in pre-weaned beef calves, disease prevention and treatment strategies are needed that decrease BRD deaths.

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Iodine Concentration in tears, milk and serum after Kelp Supplementation

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Objectives: Anecdotal reports claim that supplementation with kelp (*Ascophyllum nodosum*) can decrease incidence of pink eye and improve udder health due to increased serum, milk and tear iodine concentrations. Therefore, the objectives of this study were: 1) To measure the increase in iodine in tears, milk and serum of cows after being fed kelp for 30 days, 2) Compare the trace mineral and thyroid status of cows before (d0) and after being fed kelp for 30 days, and 3) To compare the growth rate of bacteria in tears (*Moraxella bovis*) and milk (*St. aureus*, *E. coli*, *St.uberis*) of cows fed no kelp (d0) or kelp (d30) in vitro.

Materials and Methods: Three healthy midlactation Holstein cows per group were either supplemented with 56g of kelp per day or not for 30 days. At day 0, 7, 14, 21, and 30 blood, aseptic milk and tear samples were collected. The samples were analyzed for iodine content. Furthermore, liver biopsies were collected on day 0 and d 30 for mineral analysis and blood samples were additionally analyzed for thyroid hormones. An inhibition test used milk and tear soaked plates from kelp fed cows (d0 and d30) as well as 1% and 7% iodine as positive and distilled water as negative control. Changes in assessed parameters within a group were assessed with sign tests to account for the dependence of the data, while comparisons of parameters between groups were conducted with Mann-Whitney U tests. The significance level was set at alpha=0.05.

Results: Unexpectedly the baseline iodine concentrations of the serum and milk of enrolled cows were higher than anticipated. Nevertheless, the kelp supplementation did increase iodine concentrations in milk and serum of cows and the serum iodine concentrations were positively correlated with milk and tear iodine concentrations. The liver mineral content did not change after kelp supplementation and also the thyroid hormone concentrations remained within the reference range. While the 1% and 7% iodine concentration inhibited the growth of assessed bacteria, none of the milk and tear samples or the distilled water showed any inhibition zones.

Conclusions: As anticipated the feed supplementation with kelp did impact the serum iodine concentration of cattle and milk, tear and serum iodine concentrations were correlated. However, the increased iodine concentration in tears did not inhibit bacterial growth in vitro. Therefore, an increased tear iodine concentration by itself is unlikely to decrease pink eye incidence.

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Objectives: The objective of this review was to identify non-antimicrobial therapies (i.e. treatments other than antibiotics) for the treatment of clinical mastitis in lactating dairy cows that have demonstrated efficacy in clinical trials, observational studies or experimental studies on mastitis models.

Materials and Methods: A systematic review was performed with eligible studies selected from CAB Abstract, Pubmed and Web of Science from January 1970 to June 2014. Publications in English or French were subsequently screened independently by 3 different reviewers for inclusion and exclusion criteria. References of each included publication were also reviewed. A total of 2,451 manuscripts were first identified after duplicate exclusion. Among the 167 papers remaining after title and/or abstract exclusion, 132 were excluded after full-text assessment to reach 35 included manuscripts. Four additional studies were included after review of the references of included manuscripts. Finally, 39 manuscripts corresponding to 41 studies were included for qualitative synthesis. Assessment of risk of bias in included studies was evaluated using the Cochrane Collaboration's tool for assessing risk of bias. Outcomes evaluated for the evaluation of treatment efficacy were resolution and recurrence of clinical signs which include systematic as well as local signs, bacteriological cure, and, finally, milk production.

Results: Among the 41 included studies, 22 were clinical trials, 18 were experimental studies (15 endotoxin-induced mastitis and three bacterial-induced mastitis), and one was an observational study. The different non-antibiotic treatments evaluated were: conventional anti-inflammatory drugs (non-steroidal and steroidal, n=14), oxytocin with or without frequent milk out (n=5), homeopathy (n=5), phytotherapeutic products (n=4), immunoglobulins based products (n=4), products used to enhance the immune system (n=3), products with bactericidal or bacteriostatic properties (n=2) and probiotic (n=2). High risk of biases was noted in most included manuscripts, with most frequent biases being blinding of participants and personnel (41% of studies), blinding of outcome assessment (38%), and selective reporting (33% of studies). Various other uncategorizable biases were also noted in 92% of the papers. Study power was also a frequent limitation and could have led to type 1 error in studies where only a positive control group was used.

Based on the studies available, homeopathic treatments of clinical mastitis should be considered ineffective. Some phytotherapeutic products or immunoglobulins based products need further investigation before conclusion on their efficacy. Efficacy of oxytocin with or without frequent milking out is inconsistent and some detrimental effects were even reported. Anti-inflammatory drugs have demonstrated potential beneficial effects in experimental models mainly on control of clinical signs (e.g. hyperthermia, depression) associated with clinical mastitis.

Conclusions: To date, no alternative therapies had consistently demonstrated efficacy for the treatment of clinical mastitis based on the resolution of clinical signs, bacteriological cure or milk production in clinical trials. Positive effects of anti-inflammatory drugs have to be confirmed in randomized clinical trials. Finally, to minimize bias, following the CONSORT statement recommendations when reporting clinical trials is strongly encouraged.

CM-038-002

A Systematic Review Of Non-Antimicrobial Treatments Of Clinical Mastitis In Dairy Cows.

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CM-038-003

Is the current use of homeopathy in agricultural practice target-oriented? - Studies from dairy farms in Germany, France and Spain -

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Objectives: The success of homeopathic treatment is not only related to the effectiveness of the remedies themselves, but depends also on the appropriateness of farm conditions (e.g. expertise in homeopathic principles, availability of homeopathic experts / remedies, storage of remedies, documentation, etc.). Hitherto there are no studies available providing information about the conditions for the use of homeopathy and the homeopathic procedures on dairy farms. The aim of the survey is to provide an initial overview on the current situation of the use of homeopathy in dairy production.

Materials and Methods: The study was conducted in France, Germany and Spain where homeopathic remedies are frequently used in dairy farm practice. Different questionnaires for farmers and veterinarians were developed by scientists and homeopathic experts (veterinarians) from the International Association for Veterinary Homeopathy (IAVH). In total, 64 farms were visited and served for applying the questionnaires and to gather information about homeopathic treatment procedures on the farms. To ensure the comparability of the data in the three countries, a standard procedure was adhered to, beginning with the stable tour and inspection of stable pharmacy followed by an interview with farmers questioned by the scientist and the homeopathic expert. Every questionnaire was evaluated individually by a scientist and each question assigned to different categories: e.g. prophylaxis, early detection of diseases or lege-artis step of a homeopathic treatment (e.g. anamnesis, selection of remedies, success control). Farmers' level of expertise was assessed by the homeopathic experts by using specific questions about their homeopathic treatment procedure. For the purpose of the evaluation, frequency distribution with or without previous categorisation and a ranking method were used for analysis purpose.

Results: The results of the questionnaires revealed that farm conditions for the use of homeopathy and homeopathic treatment procedure itself were very heterogeneous and differed considerably between farms and countries. The on-farm assessment of the conditions showed inter alia: poor hygiene (33% of all farms used boxes for both diseased and calving animals without implementing some kind of cleaning and disinfection) or non-compliance of specific recommended storage instructions for homeopathic remedies by 40% of all farmers. Early detection measurements such as Body Condition Scoring (14% of farms) or California Mastitis Test (20% of farms never used it) were also rarely or not performed. Most farmers only had a poor level of awareness of the principles of homeopathy (22% of farmers were rated "very poor", 51% "poor" and 27% "moderate"). The use of homeopathy by farmers was often illegal by making use of human products regularly without rededication by a veterinarian (88% of farms) or by use of forbidden substances (Colchicine and/or Aristolochia were found on 11 farms). Moreover, treatments were accompanied by insufficient treatment documentation (52% no, 31% partial, 17% of farms made a complete documentation). The studies revealed that no uniform treatment procedures in the use of homeopathy existed. The results indicate that a homeopathic lege-artis treatment of diseased food-producing animals is missing whereas each farmer seems to have developed his/her own homeopathic treatment strategy without providing evidence with respect to the success of treatment.

Conclusions: The effectiveness of homeopathy cannot be limited to the

efficacy of remedies in a standardised clinical trial. Furthermore, for a target-oriented treatment also appropriate farm conditions have to be implemented. The heterogeneous situation in farm practice makes it nearly impossible to predict the outcome of a homeopathic treatment. The reasons for the heterogeneous use of homeopathy are manifold, but without a monitoring of treatment success on the farm level and the implementation of a homeopathic lege-artis concept, an un-aided use of homeopathy could create unnecessary suffering for diseased animals.

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CM-038-004

Efficacy of homeopathy in livestock concluded from peer-reviewed publications from 1980 to 2014

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Objectives: The use of homeopathy in livestock is widespread and becomes more and more popular in the context of the general aim to reduce the use of antibiotics, especially in food-producing animals. The efficacy of remedies is decisive for its use in diseased animals in respect to animal health and welfare and consumer safety. Therefore, a systematic review of peer-reviewed publications has been conducted to assess the efficacy of homeopathy in cattle, pigs and poultry. The main focus was on studies dealing with homeopathic remedies which could possibly replace antibiotics in the case of infections.

Materials and Methods: The search for scientific publications was performed between February 2014 and June 2014 in multiple databases with search terms defined according to the PICOS approach. Study descriptions were assessed according to various criteria like species, diseases, applied methods, remedies, study design and outcome. Only publications in peer-reviewed scientific journals and doctoral theses were taken into account. The considered studies addressed the efficacy of homeopathic drugs in cattle, pigs or poultry in production diseases under European or comparable conditions. The time frame of publications considered was from year 1980 to 2014. Accessible languages were English and German. The search and filtering process was performed on the basis of the PRISMA guidelines to ensure a transparent and complete reporting and evaluated by the same person. The following information were extracted from each study and further evaluated: author, publication year and source, research body, species, farming system, purpose of application, disease in focus, inclusion and exclusion criteria for experimental animals, diagnostic method and diagnosing person, used remedy, as well as origin, ingredients and potency of the remedy, producer of remedy, way of administration, study design and control groups, methods of measurement and outcome of the study.

Results: 62 publications were identified which met the given criteria (60 individual clinical trials and 4 summarising reports). The publications focussed mainly on cattle (n=40 trials) while only a small proportion dealt with pigs (n=13) and poultry (n=7). 30 studies showed a significantly higher efficacy of the homeopathic remedy in comparison to a control group, whereas 24 showed no medicinal effect and 6 had an inconclusive or not significant outcome. Homeopathic remedies with a low potency were more likely to show a positive effect than highly diluted remedies. Trials performed in a single- or non-blinded randomized-controlled-trial (RCT) or observational trial more likely resulted in a positive outcome

than a double-blinded RCT. Cure rates when comparing treatments with conventional remedies, homeopathy or placebo showed a wide range while the nature of the remedies did not seem to make a big difference. Within the considered studies no repetitions were conducted concerning the use of the same remedy administered to the same species with a comparable medical indication or under comparable conditions.

Conclusions: A general potential concerning the efficacy of homeopathy can be seen but no trial has been repeated in a comparable manner. Thus, no reproducibility of the efficacy of a certain remedy has been proven and cannot be claimed without providing evidence within the specific farm conditions. It cannot be recommended to use homeopathy in order to replace or reduce antibiotics unless evidence of efficacy is reproduced by RCTs under modified conditions. Otherwise, use of homeopathy risks being blamed for increasing health and welfare problems instead of reducing them.

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CM-038-005

In vitro activity of crude extract of Mexican lemon (*Citrus aurantifolia*) on *Staphylococcus aureus* isolated from Small holder dairy herds

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Objectives: Oxacillin / methicillin resistant (ORSA / MRSA) *Staphylococcus aureus* has been identified in family production dairy herds which is considered as a public health risk, because of the increasing resistance to antimicrobials, therefore raising the need to seek for new alternatives in the treatment and prevention of mastitis caused by this pathogen in dairy cows. The objective of this study was to evaluate the in vitro activity of crude extracts of Mexican lemon peel (*Citrus aurantifolia*) against *S. aureus* strains isolated from dairy cows with subclinical mastitis in smallholder dairy herds.

Materials and Methods: Ninety four *S. aureus* strains were isolated from small dairy herds to study in vitro sensitivity tests to Lemon crude extracts using lemon peels on a 20% weight/volume dry matter basis using distilled water and ethanol as solvents. 1/10 serial dilutions were used to evaluate the in vitro antimicrobial activity on *S. aureus* (0.5 MacFarland). In vitro test was run by diffusion method in Mueller Hinton agar; 10 µl dilutions (25ug/mL w/v) were run by impregnated unidiscs with crude extract, the plates were incubated at 37 ° C for 24h. The inhibition zone was measured and expressed in mm according to the inhibition diameter zone which was analyzed by one way ANOVA test (P<0.05). Control tests were run using 1µg oxacillin unidiscs against sensible and resistant *S. aureus* strains. *S. aureus* ATCC 43300 was the positive MRSA control strain used to test the crude extract.

Results: The results of the activity of the extract of *C. aurantifolia* showed antimicrobial activity against *S. aureus* isolates with inhibition zones of 2 to 5 mm when compared to *S. aureus* ATCC 43300 as the control strain. All the isolations showed different in vitro sensitivity to

the crude extract (p<0.05), when using 1/10 and 1/20 extract dilutions, showing its antimicrobial in vitro effect in comparison to oxacillin unidiscs. *C. aurantifolia* crude extract 1/20 dilution showed reduced in vitro antimicrobial effect over *S. aureus* isolations and control strains when compared to 1/10 dilution.

Conclusions: The results suggest a potential antimicrobial activity of the extract of *C. aurantifolia* which could have a potential application in clinical research to evaluate phyto compounds and their antimicrobial effect on *S. aureus* in dairy cattle.

Comments: The results pose a positive expectation for the isolation of new phytocompounds with antimicrobial activity when used as teat dip disinfectants or antimicrobial drugs in dairy cows.

DH-024-001

Predicting periparturient diseases based on prepartum dry cow behaviourAnouar Belaid ¹Maria Rodriguez-Prado ¹Sergio Calsamiglia ^{1,*}¹Servei de Nutricio i Benestar Animal, Univeritat Autonoma de Barcelona, Bellaterra-08193, Spain

Objectives: The transition period in dairy cows is associated with a peak incidence of diseases (hypocalcemia, ketosis, retained placenta, displaced abomasum, metritis and mastitis). These diseases have a negative impact on the economy of farms. Preventive treatments (anionic salts, calcium boluses, monensin, dietary additives,...) to the dry group are often successful but expensive. The objective of this trial was to test a new pedometer system to assess if the prepartum behavior is a good indicator of the risk of periparturient diseases in dairy cows to allow for directed animal-specific preventive treatments.

Materials and Methods: A new pedometer system (Track a))) cow, ENGS System innovative dairy solutions, Israel) that monitors cow steps, lying and the standing time, swaps from laying to standing, and access to the feedbunk (number of times and time per access) was used in a commercial farm with 1,200 dairy cows in Lleida (Spain). Dry cows were located in a compost-bedded barn with more than 30 m²/cow and provided with a pedometer at least 21 days prior to calving. Behavior data from a total of 129 calvings were recorded and the incidence of postpartum pathologies recorded. The normal behavior of dry cows, and the association between prepartum behavior and specific diseases was explored. Data analysis was performed with SAS software (version 9.1).

Results: On average, dry cows spent 8.4 h standing, 12.3 h laying, changed positions from laying to standing 12.1 times/day, and did 1913 steps. They attended to the feedbunk 9.4 times and spent a total of 3.3 h (average of 21 minutes each access time). When cows were sick in the postpartum (for any disease), there was a reduction in the number of visits to the feedbunk (9.4 vs 8.0 times), in the time at the feedbunk (200 vs 129 minutes), in the number of swaps from laying to standing (12.1 vs 10.1) and in the number of steps (1913 vs 1386); and an increase in the laying time (735 vs 840 minutes). The pattern of changes in these indicators were unique and specific for each disease. Cows with DA walked less and reduced the number of visits to the feedbunk, and increased the laying time. Cows with metritis reduced the number of visits and the time in the feedbunk, reduced the number of steps and increased the standing time. Cows with hypocalcemia reduced the number of visits and time spent in the feedbunk, reduced total steps and reduced the number of swaps from laying to standing, and increased the standing time. Cows with retained placenta reduced the time at the feedbunk and increased the standing time. Cows with mastitis increased laying time. All these changes in behaviour were observed at least 21 days before calving, providing ample time for taking preventive actions.

Conclusions: A new generation of pedometers can be successfully used to identify changes in dry cow behavior that are associated with an increased risk of specific periparturient diseases, which may help in the application of animal-specific preventive treatments

DH-024-002

What really matters in herd health advice? The use of in-depth, qualitative interviews to investigate the efficacy of current advisory strategies.Alison Scrase ^{1,*}David Main ¹Anne Haase ²Helen R. Why ¹Emma Roe³Kristen Reyher ¹¹School of Veterinary Sciences, ²School for Policy Studies, The University of Bristol, Bristol, ³Geography and Environment, University of Southampton, Southampton, United Kingdom

Objectives: Herd health planning is a critical component of modern dairy farming, yet recent data (Bell et al. 2006) suggests negative views on this process are common and implementation of recommendations is often poor. What, then, really matters in this process, and how can it be enhanced? To ensure 'agreement and common purpose' (Sibley 2006), an in-depth understanding of both veterinarian and farmer perceptions is critical. This study is a qualitative approach to understanding this context, with the objective of revealing critical insight into what is the nature of, and what influences, veterinary advice and communication with farmers on herd health.

Materials and Methods: Farmers (n=14) located in the South West of England and their respective vets (n=10) from six South West practices were recruited via a UK retailer. Each farm was visited during a routine fertility consultation, and conversations between veterinarians and farmers were audio recorded. After completing the consultation, farmers and veterinarians were interviewed in an in-depth, semi-structured format to investigate perceptions and impacts of positive/negative communication, perceptions of veterinarian and other advisory roles on farm and attitudes towards motivation and compliance to advisory recommendations, particularly those relating to lameness and mastitis (mean interview duration 53.7 minutes).

Interview data was transcribed and organised/analysed thematically using the template methodology described by King (2004), to allow the comparison of farmer and veterinarian perspectives within this context. A sample size of 24 interviews was expected to provide sufficient variation to reach data saturation in thematic analysis, in the context of similar qualitative work in this field (Horseman et al. 2014).

Results: Preliminary data suggest that advisory behaviours on-farm, both from the veterinarian (giving advice) and farmer (seeking/receiving/ implementing advice) are influenced by factors such as the farm complexity (history, structure, workers, priorities, capabilities), knowledge and experience of both parties, the role of external advisors, and the professional and personal relationship established between veterinarian and farmer. These factors are in turn underpinned by a host of values and attitudes, such as those relating to disease processes, implementing change, the dairy cow and the veterinary role.

Conclusions: The efficacy of a herd health plan rests not only in the recommendations themselves, but in engaging farmers in plan delivery. Interview data suggest that reaching 'agreement and common purpose' is a complex phenomenon that emerges through the interaction of many personal and practical factors, not merely the content of the programme delivered. Consideration of these results (in full detail) could therefore offer those working in advisory contexts the opportunity to enhance their delivery of herd health plans and increase the potential for successful implementation of advice on farm.

DH-024-003

"KOers": A novel online data collection tool to support transition cow managementG Hofste ¹E Nijenhuis ¹M van den Berg ¹S Boersema ¹T Westerhout ¹J de Boer ¹J Lievaart ^{1,2,*}¹Dairy Consultancy, Cowcept, Loppersum, Netherlands, ²School of

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Objectives: Programs to support data collection in transition cow management are widely discussed over the last decade. Pitfalls of most current programs are; bias in data collection, how to engage farmers, inflexible systems and how to underpin day to day management.

To overcome these issues, we developed a program called "KOers" which records online various data on transition cows via fixed protocols. Our first objective was to improve data collection for early detection and intervention in transition cow management. The second objective is to add value to the regular herd health visits performed by dairy practitioners.

Materials and Methods: The software is written in a way that the farmer can choose between various components to be included (eg. udder health, lameness, reproduction, ketosis), and which parameter(s) per component have to be recorded and at which frequency. Data can be collected either by farmers and their coworkers or by veterinarians directly online via an I-pad. At the initial phase the program has been implemented on 13 Dutch dairy farms for 18 months (2014-2015) at a large dairy practice in the Northern part of the Netherlands. All farmers participated in a weekly schedule and all components of udder health, lameness, reproduction and ketosis were included. Data were collected by three practitioners. To assess the results of the program, herd health parameters before and after 12 months after the program KOers had been implemented were analyzed.

Results: The average herd size of the 13 farms was 123 vs. 119 cows at the start and the end. The Daily Dosage (DD) of antibiotics use per cow per year did decrease from 4.3 to 2.2 on average. The average Bulk Somatic Cell Count (BMSCC) did not change (155* 103 vs. 152*103). The number of cases of clinical mastitis was on average 14.1% at the start and 12.5% after the tool was implemented for 1 year. Lameness cases did decrease from 20.1% to 12.6%. The inter-calving period decreased from 418 days to 403 days on average including a decreased number of inseminations per cow (2.2 vs. 1.9).

Conclusions: From our own and the farmers experience with the KOers system, we believe it's a valuable additional tool to successfully monitor the transition cow period and add value to the regular herd health visit of dairy practitioners. The objectives have been met and the program will be enrolled on a large scale on Dutch dairy farms.

DH-024-004

Use of Acute Phase Proteins for Monitoring Cow Health and Productivity in Dairy Cattle in the UK

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Objectives: Analysis of blood biochemistry (e.g. metabolic profiles) is used as a method for the routine evaluation of nutritional adequacy in dairy herds with the purpose of identifying production-limiting factors. However subclinical disease may also be present within the herd, and may confound the interpretation of metabolic profile analysis. The aim

of the present study was to 1) evaluate the diagnostic value of using Acute Phase Protein (APP) assays in metabolic profile screening programmes and 2) examine the association of APP with clinical disease and production parameters in dairy cows.

Materials and Methods: Blood samples were obtained from 12 commercial dairy herds as part of a routine nutritional monitoring programme using metabolic profiles. Aliquots of serum were frozen and stored at -20°C, and subsequently analysed for the APP haptoglobin (Hp) and Serum Amyloid A (SAA) using a haemoglobin binding method and an ELISA respectively.

Individual cow somatic cell count (ICSCC) data was obtained for the two milk recordings closest to the actual date of blood sampling from 9 farms. Appropriate disease records were obtained from 9 farms, and any disease occurrences within 100 days of the blood sampling date were recorded along with the date they occurred. Relevant fertility data recorded from all 12 farms included date of 1st service, number of services, and date of successful service and whether the cow was back in calf by the end of that lactation. Three farms were organic.

Data was analysed using mixed effect models with farm identity entered as a random effect to account for intra-farm correlation, and system (standard or organic), lactation number (primiparous or multiparous) and group (EARLY, MID or DRY) added as fixed effects. Data was analysed either as raw data, or binomial data (using values above or below SAA and Hp thresholds) where Relative Risk was calculated with 95% CI.

Results: A total of 388 blood samples were received from individual cows from the twelve farms. Using a threshold of 0.02 g/l, 155 cows (40%) had elevated Hp results. Using a threshold of 24 mg/l, 165 cows (42.5 %) had elevated SAA results. Hp and SAA were significantly positively correlated ($r_s=0.377$, $P<0.001$), although only 64.5% of the samples were in agreement (ie. SAA and Hp were either both high or both low relative to thresholds).

There were 75 disease events recorded in the cows sampled, with 39 separate cases of mastitis, 15 cases of metritis or endometritis and 10 cases of lameness. There was no significant association between elevated APP and all disease occurrences. However the Relative Risk of a cow having a lameness event if she had a high SAA result was 6.203 (95% CI: 1.363 – 28.24). Using either the actual ICSCC value or a 200,000 cells/ml threshold, there was no significant association between APP (either Hp or SAA) and ICSCC.

There was no significant effect of elevated APP (either Hp or SAA) on production, measured by total lactation yield, 305 day lactation yield or milk per day of lactation. The Relative Risk of a cow being culled if she had a high Hp result was 1.506 (95% CI: 1.086 – 2.088).

Cows with elevated Hp levels received more services ($P=0.038$), took an average of 21.5 days (± 9.62 SEM) longer to get in calf ($P=0.025$), and had a 100 day in-calf rate of 20.8% compared to those cows with low Hp levels (33.2%)($P=0.007$). The Relative Risk of a cow being in calf at 100 days in milk if she had an elevated Hp value was 0.616 (95% CI: 0.432 – 0.878).

Conclusions: This study of 388 cows from 12 different dairy farms showed that a significant proportion of cows had elevated Hp and SAA levels (over 40%). There was a significant positive association between SAA and lameness occurrence. The main findings showed a significant association between elevated Hp levels and an increased risk of culling and reduced fertility parameters, which illustrate the potentially harmful effects of inflammation and subclinical disease on dairy cow productivity. Inclusion of APP analyses may provide a useful additional tool for the monitoring of dairy cow health and welfare.

DH-024-005

On farm cattle mortality and related risk factors in Estonian dairy herds

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Objectives: Increasing dairy cow mortality has been reported in several countries all over the world. Mortality of dairy cattle causes financial loss for the farmer and is connected to animal welfare. The aim of this study was to determine the mortality risk and rate, as well as risk factors for on-farm mortality (unassisted death and euthanasia) in cattle of Estonian dairy herds in years 2013 and 2014.

Materials and Methods: Data was collected from Estonian Agricultural Registers and Information Board. Mortality risk and rate in years 2013 and 2014 was estimated based on data consisting cattle from all dairy herds (more than 75% of dairy breed cows within herd) in Estonia, including 274,327 animals from 2,631 farms, and 283,545 animals from 2,321 farms for 2013 and 2014, respectively. Two years study period was used for screening multivariable associations between potential risk factors and mortality including animals present in dairy herds at the start of the observation period (1st of January 2013) and animals born during years 2013 and 2014. The dataset included 362,683 animals from 2,614 farms. All statistical analyses were performed using STATA MP version 14 (StataCorp LP, College Station, USA). Factors of interest were age group, breed, gender, herd size and region. Multivariable Weibull proportional hazard models with farm as random effect were composed for detecting significant associations between potential risk factors and on-farm mortality.

Results: In 2013 there were 12,834 deaths and euthanasias, number of animal-years was 209,474, mortality risk 4.68% and mortality rate 6.13 per 100 animal-years. In 2014 13,854 deaths and euthanasias occurred resulting mortality risk of 4.89% and mortality rate 6.37, number of animal-years in the analysis was 217,527. The increase of cattle mortality in dairy herds in 2014 compared to 2013 was statistically significant ($p < 0.05$). Statistically significant interactions were found between age and gender, breed and gender as well as between herd size and region. The hazard of on-farm mortality differed significant between gender and age groups ($p < 0.001$), being highest among females aged <12 months (baseline group with HR = 1), followed by males in the same age group (HR = 0.87, 95% CI 0.72; 1.06, $p = 0.166$). Consistent with all age groups males had lower hazard of dying compared to females. The hazard of failure was significantly lower among female animals from Estonian Red breed (HR = 0.80, 95% CI 0.76; 0.85, $p < 0.001$) and category of 'other' breeds (HR = 0.69, 95% CI 0.59, 0.81) compared to female Estonian Holsteins (baseline group). The risk of on-farm death and euthanasia among male Estonian Holstein and male Estonian Red cattle did not differ significantly from the baseline group. The hazard of mortality was highest among herds with 100-300 cows located in North of Estonia (HR = 2.00, 95% CI 1.72; 2.31, $p < 0.001$) and in herds with 300-600 cows in South of Estonia (HR = 1.87, 95% CI 1.67; 2.08, $p < 0.001$) compared to smallest farms (<100 cows) positioned in South.

Conclusions: This is the first study demonstrating the mortality rate in Estonian dairy cattle population. On-farm mortality has increased between years 2013 and 2014. There are considerable differences between age, breed and gender differences contributing to mortality risk. The effect of herd size on cattle mortality was influenced by the region. This study is a good starting point for more detailed research that could identify significant factors associated with cattle mortality that could be used to improve animal welfare and farmer's profitability.

DI-029-001

Calf umbilical disorders : evaluating correspondence between ultrasound images, abdominal palpation and subsequent surgical observations.

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Objectives: Calf umbilical disorders cause complications and important economic loss. An accurate diagnosis is mandatory to make an adequate prognosis and good therapeutic choices. In this study, we evaluate the efficiency and the interest of this tool in omphalophlebitis diagnosis when compared to abdominal palpation and subsequent surgical observation.

Materials and Methods: 37 calves were referred at the farm animals hospital of the Toulouse vet school (ENVT) for a clinical suspicion of omphalophlebitis in 2015. All of them were carefully examined at arrival. Physical examination included abdominal palpation. Physical examination was followed by ultrasonography of the abdomen, using a 1-8 Mhz sectorial probe. Subsequent surgery was performed in all cases, leading or not to a confirmed diagnosis of omphalophlebitis. 28 calves presented really omphalophlebitis (the more frequent cause of error was hernia, followed by abscesses outside of the navel).

Results: Palpation is of great use when calves are younger than 2 weeks old. Palpation is of no use when the calves are older than 3 months old.

In case of infection of the umbilical vein, ultrasonography enables : Se = 1, Sp = 1, PPV = 1, NPV = 1 ; in case of posterior disorder (urachal or umbilical arteries) : Se = 0.86, Sp = 1, PPV = 1, NPV = 0.85.

Moreover, even if the abdominal palpation permits an accurate diagnosis, ultrasonography provides useful information (nature of the vessels involved, size of the lesions, signs of peritonitis, involvement and lesion of the urine bladder...).

Conclusions: Ultrasonography is more and more used by veterinary practitioners. Trained vets can perform easily and promptly ultrasonography in calves suffering from omphalophlebitis. The performances of ultrasonography in this field of medicine are quite good, superior to the physical examination alone. Because ultrasonography allows the detection of unrevealed lesions useful to know before surgery, this imaging technique could be more and more used in the field.

DI-029-002

Comparison of ultrasonographic findings obtained using 5 MHZ linear and microarray scanners in cattle with lung and pleural pathology.

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Objectives: Modern portable ultrasound machines provide the veterinary practitioner with an inexpensive, non-invasive tool with which to examine the pleural surfaces and superficial lung parenchyma of cattle with suspected respiratory disease however published studies have used 5MHz microarray scanners when most veterinary practitioners use 5MHz linear scanners everyday for trans-rectal early pregnancy diagnosis. The

objective of this prospective study was to determine whether a 5MHz linear scanner significantly altered the diagnosis and prognosis of lung and pleural pathologies

Materials and Methods: Few veterinary practitioners routinely examine the lungs and pleurae of cattle with suspected respiratory disease. Ultrasonographic examination of cattle with suspected respiratory disease was undertaken over a three year period using both 5MHz linear and microarray scanners with results recorded as mp4 files on computer using Elgato software. Cases that did not respond to treatment were euthanased for welfare reasons and necropsied. Follow-up of cases was undertaken after three months by telephone.

Results: Growing cattle (6) and adult cattle (16) with chronic suppurative pneumonia gave similar results with respect to the dorsal margin of lung pathology and ultrasonographic appearance of lung pathology; no ultrasonographic abnormalities were observed in a similar number of healthy control cattle using either scanner head. Lobular lung pathology was observed dorsally in four cows with chronic suppurative pneumonia represented by numerous hypoechoic columns extending 2 to 6 cm into the lung parenchyma. This sonographic appearance abruptly changed as the probe was moved ventrally to large hypoechoic areas corresponding to lung consolidation with the echogenic appearance of liver (hepatoid change). This abrupt change from normal lung to consolidation was observed in all other cases. The dorsal margin of a pleural effusion in one cow was readily identified using both scanners as an anechoic area which increased in depth to 16 cm as the microarray probe head was moved ventrally but measurement of the effusion with the linear scanner was only 6-7 cm due to the limited field depth. Similarly, a pleural abscess extended to 15 cm from the chest wall (microarray probe) but a maximum depth of 6-7 cm was recorded using the linear probe.

Conclusions: 5MHz linear probes can be successfully used to identify lung pathology such as chronic suppurative pneumonia and measure its dorsal margin which is the critical factor in prognosis of this disease process. The depth of pleural effusion cannot be measured beyond the 9-10 cm range of a linear probe (less 2-3 cm of chest wall) but can be estimated by how far dorsally it extends. While the area of pleural surface affected by an abscess can be determined, its depth beyond 6-7 cm cannot be measured. In this study the limited depth of a linear probe did not affect the diagnosis nor prognosis of the diseases reported.

DI-029-003

Prevalence of lung lesions using thoracic ultrasonography in pre-weaned calves from dairy herds in Québec, Canada.

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Objectives: Thoracic ultrasonography and observation of lung ultrasonographic consolidation has been recently developed as a fast and non-invasive way to assess bronchopneumonia associated lung lesions. The objective of this cross-sectional study was to assess the prevalence of lung consolidation in pre-weaned calves in dairy herds.

Materials and Methods: Thirty nine dairy herds were randomly selected in the client list of the ambulatory clinic of the Université de Montréal to participate to this cross-sectional study conducted during the summer 2015. A questionnaire focusing on calf feeding and raising practices was completed with the producer at the day of the visit. Each

calf (min 6 calves, max 12) was assessed using a complete thoracic ultrasound examination (7.5 MHz linear probe, I-scan, Draminski, PL) focusing on the maximal depth of consolidation observed. Two different cut-offs for identifying a calf with consolidation were selected. A high sensitive definition of consolidation was to have at least one site with a consolidation depth ≥ 1 cm (Cons1). A more specific definition was used defining a consolidated calf when at least one site with a consolidation depth ≥ 3 cm was found (Cons3). Descriptive statistics were calculated.

Results: A total of 318 pre-weaned calves from 39 different herds were enrolled in this study. The median number of lactating cows in these herds was 80 (ranged from 55 to 280). The maximal quantity of milk replacer volume offered to calves during the pre-weaning period varied from 4 to 16L per day (median=8L). The herd-level prevalence of Cons1 varied from 0 to 80.0 % (median 16.7%; 25th percentile 10.4%; 75th percentile 41.2%; 90th percentile 60.0 %). The herd-level prevalence of Cons3 varied from 0 to 70% (median 8.3%; 25th percentile 0%; 75th percentile 20.8 %; 90th percentile 33.3%).

Conclusions: Despite the fact that this study was performed in a period with a relatively low anticipated incidence of enzootic pneumonia (ie. Summer time), ultrasonographic evidences of lung lesions were commonly found in dairy herds. Interestingly, the prevalence of these lesions was highly variable between herds.

DI-029-004

Interest of ultrasonography for the diagnostic of congenital heart defects in cattle: 12 years of experience 2003-2015

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Objectives: In routine cattle practice, heart congenital defects are never the reason spontaneously expressed by the farmer to call his vet. Heart congenital defects account for 3% of the whole congenital defects in cattle. Among heart congenital defects, abattoir surveys and/or description from veterinary hospital case reports reported that ventricular septal defect (VSD) is the most frequent (42-65%). The aim of this presentation is to describe the clinical, ultrasonography and necropsy findings of the cases of heart congenital defects found during a 12 years period in the Clinics for Ruminants of Oniris.

Materials and Methods: During this period, clinical, ultrasonography and necropsy data were available for 100 cases.

The ultrasonographic examinations were made while the animals were standing using a 2.5 MHz phased array transducer (Mylab Five Vet, HospiMedi France) in 2-D Mode. The heart was ultrasonographically examined on the right cranial parasternal side in six positions as classically described in small animals. VSD were easily and systematically observed. The distinction between other malformations, Patent Ductus Arteriosus (PDA), Aortic Stenosis or complex congenital cardiac defect like Tetra/Pentalogy of Fallot, was not always easy to make especially regarding the identification of all defects.

Results: The clinical observations led to the following patterns:

- Clinical suspicion based on clinical signs only related to cardiovascular system in 25 cases
- Clinical suspicion associated with growth retardation in 20 cases
- Clinical suspicion associated with chronic respiratory disorders for

which antibiotics and/or NSAIDs were ineffective in 20 cases

- Clinical suspicion associated with multiple malformations (head, eyes, bones) in 20 cases.
- Clinical suspicion and/or endocarditis in 15 cases.

At necropsy:

- Ventricular Septal Defect (VSD) was noticed in 86 cases
- Atrial Septal Defect (ASD) was noticed in 22 cases
- Tetra and Pentalogy of Fallot in 9 cases
- Other malformations (Patent Ductus Arteriosus, Aortic Stenosis,...) in other cases
- Endocarditis associated with VSD, in 15 cases, only in animals older than 2 years.

Conclusions: Our results confirm that VSD is the most frequent heart congenital defects in cattle, but less frequently in association with other defects or associated/secondary lesions such as endocarditis. Our results highlight also that heart congenital defects must be suspected in clinical pattern such as growth retardation or chronic respiratory disease. In that specific case, ultrasonography is a sensitive and specific method for both investigating diagnosis and making a prognosis. Ultrasonography could also help to more precisely define phenotypes to investigate putative genomic patterns underlying these defects.

DI-029-005

Ultrasonography of the rumen wall as a potential diagnostic tool to diagnose of Subacute Rumen Acidosis (SARA) in fattening bulls.

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Objectives: Importing bulls is a common practice in intensive system of beef cattle in Italy. The acclimation period following the restocking is a critical phase, where animals change from a pasture diet to a high concentrate ration increasing the risk of Acidosis or Sub Acute Ruminant Acidosis (SARA).

Ruminal mucosa thickening in response to volatile fatty acids concentration and rumen pH are well established; rumenitis are a frequent sequel to acidosis.

The aim of this study was to evaluate the viability of transabdominal ultrasonography of the rumen mucosa as a suitable, non-invasive diagnostic tool to identify beef cattle affected by SARA.

Materials and Methods: One hundred– fifty four bulls of Charolaise breed were selected in 2 farms of 2500 feedlot cattle in the North-East, Italy. The animals were imported from France with an average weight of 424.5 \pm 33.4 kg and an average age of 17.0 \pm 1.9 months.

The arriving beef cattle were placed on a fibrous acclimation diet for 5 days prior to the total mixed ration (TMR) high concentrate growth diet. Stall had a separate feed bunk and watering point. Dry matter intake (DMI) mean values were recorded for all animals during the period of study (DMI: 18 \pm 1.5 Kg per animal; DM: 9.78 \pm 0.8 per animal).

Individual rumenocentesis were carried out 4-6 h after feeding, 10 days after housing. Rumen fluid was collected using a 13G 105-mm needle and a 50ml syringe from a disinfected area in the left flank, from the

ventral sac of the rumen, approximately 15–20 cm caudal and ventral to the costochondral junction of the last rib. 15 ml of rumen fluid were collected from each animal.

Ruminal pH was measured immediately after sampling using a portable pH meter.

Ultrasonographic examination of the rumen wall was conducted on the same point used for rumenocentesis with an 6.6MHz convex transducer (acoustic windows = 7cm).

One way Analysis of variance was used to detect differences in measured parameters. Regression analysis (R2) and Pearson's correlations were performed with thickness of the rumen wall, rumen mucosa as dependent and ruminal pH as independent variables. Receiver Operating Characteristics Analysis (ROC Analysis) were conducted to identify suitable cut-off for thickness of the rumen mucosa ultrasonography.

Results: The ruminal fluid pH findings of the 154 young bulls showed significant statistically differences ($P < 0.001$): $pH < 5.5$ (29 animals); $5.5 < pH < 5.8$ (35 animals); $pH > 5.9$ (90 animals).

The regression analysis conducted on ruminal fluid pH and total ultrasound thickness of rumen wall and rumen mucosa showed respectively $R^2 = 0.5637$ and $R^2 = 0.5895$.

Pearson's analysis showed good interaction between pH and total ultrasound thickness of rumen wall (-0.700 ; $P < 0.0001$) and rumen mucosa (-0.7921 ; $P < 0.0001$).

Roc analysis revealed that cut-off values for the mucosal thickness of 7.8 mm had sensitivity of 0.85 and specificity of 0.81 to identify animals with a rumen fluid < 5.8 at 4-6 h after feeding.

Conclusions: Our results show that after 10 days from the housing 41.55% of the fattening bulls had a rumen pH lower than the threshold value of 5.8. This lowering of the pH may be attributed to the sudden change in diet and the difficulty that the bulls may have to adapt.

The thickening of the ruminal mucosa is also well correlated with the lowering of ruminal pH.

Transabdominal ultrasonography of the rumen mucosa has the potential to be a suitable diagnostic tool to identify fattening bulls affected by SARA. This study provides encouraging preliminary data warranting further examination and validation in fattening bulls.

DI-029-006

Ultrasound findings of common pathologies of the female genital system of small ruminants

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Objectives: Dysfunctions of the female genital system in small ruminants are common findings in the buiatrics practice. This study reports the prevalence and ultrasound appearance of some common reproductive tract pathologies of small ruminants under tropical conditions in Brazil.

Materials and Methods: A three years' retrospective study of ultrasound data collected during pregnancy diagnosis and evaluation of the

reproductive tract was carried out from September 2012 to September 2015 at Rio de Janeiro State. A total of eight sheep flock and four dairy goat flock raised under extensive and intensive management systems, respectively, were used in the study. A ultrasound device (Sonoscape S6®, Sonoscape, Yizhe Building, Yuquan Road, Shenzhen, China) coupled to a linear transducer of 7.5 MHz (transrectal) or 5 MHz convex (transabdominal). B-mode and Color Doppler-mode ultrasound tapes were recorded and evaluated by the same operator (MB). The chi-square test was adopted for frequency comparison ($P < 0.05$).

Results: Ultrasound data were collected from 1453 examinations (60 per cent ewes and 40.0 per does). Reproductive disorders were detected in 137/1453 (9.5%). Of these, hydrometra was the major occurrence (3.7%; 53/1453), followed by aseptic resorption of the embryo/fetus (2.3%; 33/1453), and recent embryony/fetal death detected by the lack of heartbeat (1.3%; 19/1453). Other findings in descending order were: cystic endometrial hyperplasia (0.6%; 9/1453), follicular cyst (0.6%; 9/1453), septic resorption (0.2%; 3/1453), pyometra (0.1%; 2/1453), hydrosalpinx (0.1%; 2/1453), and single cases of ovarian tumour, macerated fetus, mummified fetus, luteal cyst, cervicitis, retained placenta, and supracervical abscess (0.07%; 1/1453). Sheep had significantly fewer reproductive disorders than goats (5.3%; 46/865 vs. 15.5%; 91/588 $P < 0.05$). In goats, there was a greater occurrence of hydrometra (8.2%; 49/588 - $P < 0.05$) followed by aseptic resorption (2.7%; 16/588). In sheep, the occurrence of aseptic resorption came first (2.0%; 17/865 - $P > 0.05$) followed by recent embryony/fetal death (1.3%; 11/865).

Conclusions: Dairy goats had significantly more reproductive tract disorders than sheep where hydrometra was the major issue. Ultrasonography provides clinically useful information relating to diagnosis, prognosis, and therapeutics.

Comments: The authors have a vast visual material on the subject. So, it is sought the acceptance of the abstract as an oral presentation under the aim to share videos and knowledge with the public at Buiatrics congress and stimulate the discussion about the topic.

DI-029-007

Traumatic reticuloperitonitis : correlation between necropsy and ante mortem clinical and laboratory data (ultrasonography, biochemistry, hematology)

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Objectives: The purpose of this study is to determine the reliability of ultrasonography in traumatic reticuloperitonitis diagnostic (TRP) in cattle admitted at the ENVT farm animals hospital, compared to the results of physical examination, blood biochemistry, hematology.

Materials and Methods: 73 cows were admitted for a clinical suspicion of TRP. Complete physical examination (pain, tachypnea, tachycardia, anemia, milk drop) , ultrasonography of the reticulum using a 3.5-5 Mhz sector probe, blood biochemistry (total proteins, fibrinogen), hematology (white and red blood cell counts) were performed. 38 (on 73) had to be euthanised or died during hospitalisation, allowing an accurate diagnosis at necropsy.

When compared to necropsy, sensitivity (Se), specificity (Sp), Positive

Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated for each ante mortem test.

Results: Physical examination alone is of poor value (Se and PPV < 0.25). Blood cell counts and physical examination are of no use too. These figures are even worse when facing chronic TRP cases (which represent the major part of cases).

Combining physical examination and ultrasonography of the reticulum leads to: Se = 0.81, Sp = 0.47, PPV = 0.65, NPV = 0.67. Combining physical examination, ultrasonography and plasmatic biochemical values leads to: Se = 1, Sp = 0.38, PPV = 0.62, NPV = 1. Ultrasonography is of great value when considering signs of peritonitis, signs of inflammation, aspect of the reticulum wall, motricity.

Conclusions: Traumatic reticuloperitonitis is a common and often severe disease (taking in account the number of cattle concerned and the induced lesions on cattle organs). This leads to productive losses and anticipated culling. That is why it is convenient to set a precise diagnostic in animal husbandry, so that the farmer can take an adequate decision concerning the future of his animal. Outside of the reproductive surveys, ultrasonography in cattle is a technic that has been developed a lot in the last few years. This study assesses the great value of ultrasonography in reticuloperitonitis diagnosis.

EDU-022-001

Developing a Producer-Centered Approach to Design and Optimize Training Programs in Antimicrobial Stewardship

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Objectives: While there have been many studies exploring the impact and effectiveness of antimicrobial stewardship programs in people, there is little data to define the components of similar initiatives in livestock production. Effective teaching encompasses complex tasks that include the planning, preparation and delivery of learning activities, in line with the expectations and needs of the learners. The objective of this study was to collect data regarding antimicrobial use, behavior and attitudes, along with information related to learning preferences of producers in Illinois with the aim of developing educational materials and delivery platforms.

Materials and Methods: Areas of strengths and weaknesses in knowledge, attitude, behavior, and practice in antimicrobial usage were identified by interviewing twenty dairy producers throughout the state of Illinois. The interview included questions regarding first line antimicrobial choices, disease indications, maintenance of medical records, existence of diagnostic and treatment protocols for common diseases, extent of veterinary involvement, understanding of the concept and consequences of extra label drug use, and familiarity with the concept of antimicrobial resistance. This data was used to identify common practices in antimicrobial use by producers, and their understanding and application of legal guidelines.

Subsequently, a questionnaire regarding producer demographics, current methods or resources used for continuing education, level of engagement in their work environment, and learning style preferences was administered to 187 livestock producers by face-to-face interviews at county fairs throughout Illinois. Survey responses were analyzed and examined for statistically significant responses based on gender, age, educational status, and level of engagement.

Results: Results from the initial survey identified key areas where educational intervention could have a significant impact: antibiotic selection/use of protocols, on-farm record keeping and extra-label drug use. Only 15% of those that responded indicated use of written treatment protocols on farm; 60% self-reported no protocols being used for treatment of disease. In conjunction, less than 50% of respondents could identify criteria that constituted extra-label drug usage (ELDU), with only 25% of producers seeking veterinary guidance when choosing to use an antimicrobial in an extra label manner.

The learning inventory demographics identified livestock producers from across the dairy (6.5%), beef (53%), small ruminant (32%) and swine (40%) production sectors, with some involved in more than one enterprise. The age range showed a bell-shaped curve centered on the 45-50 years, ranging from 18 to >71 years. More than 50% of respondents had completed some form of post-secondary education, and there was equal representation from laborers, animal health care providers and financial decision makers. Internet, magazines, and veterinarians were identified as the most common animal health information sources, and most producers identified themselves as strongly engaged in their work.

The learning styles of the participants could be classified into four learning categories, namely pragmatists, activists, theorists, and reflectors. While many producers demonstrated characteristics of multiple learning styles, there were clear patterns of preference associated with specific demographics.

Conclusions: The study showed areas of strength and weakness in knowledge, attitude, behavior and practice in antimicrobial usage in producers. To enhance the effectiveness of any training program, the principles of education must be used to design and deliver learning materials. Identification of weaknesses in using treatment records and protocols, and understanding ELDU, were identified as key components of an antibiotic training program. Understanding the prevalent learning styles, and their association with producer demographics, will be paramount in designing effective training activities and a healthy learning environment for livestock producers.

EDU-022-002

Abattoir feedback from veterinary students as a tool for herd health improvement

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Objectives: According to EU legislation, conditions found at meat inspection are to be recorded by the Official Veterinarian (OV) if they "might affect public or animal health, or compromise animal welfare". Findings must be communicated back to the producer and their veterinarian. This flow of information is frequently used in the pig and poultry industry, but less accessed by cattle producers.

Bristol University's abattoir offers slaughter and butchery services to local farmers. A pilot project involving students on rotation investigated if and how inspection results are used to improve herd health, and how feedback may add to producers' knowledge.

Materials and Methods: Case reports produced by veterinary students during rotations were collected throughout 2015. These reports detail the results of an inspection performed by each student for one batch of animals. Each student presents their findings orally, and after discussion on what feedback will be useful for the producer, a written report is submitted and marked.

Each case report contains information collected at ante-mortem inspection (animal ID, producer details, loading times, clinical presentation, cleanliness, Food Chain Information) and from welfare assessment (stunning method, stun parameters, stun-to-stick times, and signs of successful stun). Post-mortem inspection is categorised into Carcass, Respiratory, Alimentary, Lymphatic, Circulatory, Skeletal, Muscular and Other. The student's findings and advice are sent to the producer, with advice to contact their own veterinary practice should they require further information.

A survey was conducted among the producers who received an advisory letter. The producers were asked whether the letter provided new knowledge, and whether they implemented any changes in their production system following the advice in the letter. Furthermore they were asked if they felt such a report added value to the service of slaughter provided by the abattoir, and if they would prefer to receive such reports also in the future.

An online survey was conducted among the students who had participated. The students were asked whether they believed this feedback was useful to the farmer, and how useful they felt it was as a learning experience.

Results: 41 case reports from cattle slaughtered at Langford Abattoir, School of Veterinary Sciences, were submitted in 2015. Most students reported no findings at ante-mortem inspection (68.3 % of reports), with the most common findings being signs of mild stress (14.6 % of

reports). All animals were found to be of adequate cleanliness. At post-mortem inspection the most frequent conditions observed were *Fasciola hepatica* (12.2% of reports), either as acute or chronic infection. Pleuritis (9.8 % of reports) and pneumonia (9.8% of reports) was seen in a few animals. However, most cattle slaughtered are young stock from local smallholders, and overall there were few disease findings observed.

Following receipt of the advisory letter, farmers have indicated that this gave an explanation for why organs had been condemned, that they had not received before. Condemnation of bovine livers for example, represent a loss of income, which warrants explanation. The abattoir does not regularly pass inspection results back to the farmers, as is the situation in many other UK slaughter plants (E. G. M. van Klink, P. G. Prestmo and A. Grist "Animal health and disease monitoring in the abattoir", *Livestock* Vol 20 (6), pp. 330-335).

Feedback from students has been overall positive, with students reporting the learning experience as useful. In particular, students indicate the benefit in having to produce a report that not only is marked by the tutors, but also gets sent to the client as a potential tool in herd health management.

Conclusions: Meat inspection results could be a useful tool for monitoring animal health on farm, especially for common subclinical conditions. There is great potential for improvement in the flow if this information, that may not have a high cost. Producers could use results together with their private veterinarian, e.g. to evaluate infection control strategies.

We aim to show students the potential which lies in this relationship between producer, veterinarian on farm and OV. We hope that new graduates who start working with food-producing animals will make use of inspection results, and that new networks may form between OVs and private veterinarians.

EDU-022-003

The Global Resource for Online Evidence-Based Veterinary Medicine Learning

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Objectives: The evidence-based veterinary medicine (EBVM) Learning Consortium, an extensive international team with a collective passion for delivering high-quality teaching, has developed a coherent web tutorial designed to introduce learners to the key concepts of EBVM: www.ebvmlearning.org. This project brought together key stakeholders and experts to identify requirements for development of EBVM modules and to create online re-usable learning material. This tutorial will provide opportunities for students and members of the profession to engage in

EBVM, and will help ensure the topic is introduced well and adopted wholeheartedly by the profession.

Materials and Methods: EBVM Learning, funded by the Royal College of Veterinary Surgeons Knowledge Target Grants initiative, brought together a respected international team expert in the field of EBVM. Since knowledge of the theory, teaching, and practice of EBVM already existed at universities, this project team was made up of experts in the field, and also represented such diverse backgrounds as clinicians, scientists, researchers and experts in many areas of the veterinary profession. Collaboration, sharing and adaptation of the many resources already developed by individuals at a number of institutions was seen as the most powerful way to meet stakeholder needs and make a significant contribution to EBVM. An open-access, online tool explaining the basics of EBVM was created by marrying engaging content with accessible and usable technical design in order to encourage and facilitate re-use in a wide variety of familiar and unexpected settings as well as a number of platforms and devices. The resource was designed to utilise best pedagogical approaches, and included formative multiple choice questions, short tasks and recommendations for further study. It is intended to be a base of knowledge bringing together the basics of EBVM into one place, and highlights numerous other links to resources and organisations that are available to further learners' knowledge about the subject. Development of the resource followed an iterative cycle which included regular review by both the core team as well as other identified stakeholders (e.g. students, practitioners, industry representatives).

Results: An open access, online tutorial aimed at making EBVM accessible to the veterinary profession worldwide was successfully developed and launched in October, 2015. The resource begins with an introductory module – the ABCs of EBVM – and is then organised into modules addressing the five key areas of EBVM:

- 1 Ask - how to formulate answerable questions
- 2 Acquire - how to obtain relevant information
- 3 Appraise - how to evaluate the available evidence
- 4 Apply - how to apply the evidence to clinical practice
- 5 Assess – how to measure the effect of any implemented changes

The tool is appropriate for students and practitioners for self-study, and it is also envisaged that the resource will be used in whole or in part as standalone teaching modules to support other EBVM teaching or continuing professional development. The resource will offer the flexibility to be re-used in a variety of teaching and learning scenarios across the sector, or for independent learning. The tutorial also comprises a freely-available series of online, re-usable learning objects (videos, quizzes, case studies, exercises, worked examples and links basket, focussing on examples from various species) that runs the gamut from introducing basic terminology to providing detailed assistance with each of the steps in turn, in language accessible to practitioners who are keen to learn these techniques. This research highlights a specific learning tool, but also recognises a number of resources that are currently available to help farm animal practitioners employ EBVM.

Conclusions: Development of this resource has already increased awareness of EBVM in the veterinary profession, and allows practitioners the opportunity to develop the skills needed to utilise EBVM in everyday clinical practice. Future aims of the project team include designing methods of cataloguing and disseminating evidence syntheses to support clinical decision-making and evidence-based veterinary practice, improving and standardising the teaching of EBVM to undergraduate and postgraduate students at veterinary schools internationally and building a worldwide community of practice in the area of EBVM.

EDU-022-004

Developing a commercial herd health service to be used for undergraduate training

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Objectives: As part of the final year clinical rotations at Nottingham Vet school, a two week "herd health" rotation was developed to be undertaken by groups of 3-6 final year veterinary students. The goal of the rotation was to teach the principles of applied epidemiology and herd health to students using dairy farms as an example whilst also providing a chargeable consultancy service to commercial dairy farms. The aims of this project were to identify whether the Nottingham Vet School Herd Health Rotation has been effective in terms of:

1. Student satisfaction
2. Client satisfaction
3. Client performance

Materials and Methods: Farms were recruited to the service through the veterinary school's network of associate practices. The rotation has been running for 5 years with each farm visited by a different student group and academic members of staff approximately every 3-6 months.

Student feedback is formally collected at the end of each rotation using a scoring system standardised across all the final year rotations as well as gathering free text comments. Client feedback is collected via the associate practice and from direct feedback from the clients. Client performance is monitored as part of the service and farm management data is collated in TotalVet (QMMS/SUM-IT) analysis software allowing benchmarking and monitoring over time.

Results: Student feedback has been strongly positive, with the rotation consistently among the top scoring clinical rotations in every area evaluated. Example free text comments are included below:

"Enjoyed this rotation much more than I thought I would as cows and stats aren't really my deal but it was great. It was really well structured with a good encouraging staff support network"

"This rotation gave our group a great opportunity...had a real impact on the herd and on the farmers business"

"Everything suddenly came together when we started looking at our farm and then discussing our findings for the farmer"

Weaknesses identified and improved mainly relate to the logistics of delivering the rotation, although additional sessions have been added to cover further topics.

Client satisfaction appears good, with clients keen to remain in the scheme and requesting visits. As in private practice, this type of service is not suited to all clients, and there was some initial "drop-out" from farmers. Some farms were not considered appropriate to remain in the service due to poor compliance or data quality.

Client performance is good and continues to improve. Some example findings are included below:

"10 cows that previously scored sound (0 or 1) have been scored lame (2 or 3) in May; these are likely NEW lameness and must be prioritised for treatment"

"the rate of new clinical mastitis cases occurring less than 30 days into lactation (i.e. cases from likely dry period origin infections) has been very well controlled following our discussions ... averaging less than 1 in 12 animals affected"

Conclusions: Whilst this service has been and continues to be refined, the authors consider the rotation a success. Student and client feedback is positive and there has been demonstrable improvement in farm performance.

This demonstrates that it is possible to provide a chargeable herd health service alongside the provision of good quality training in herd health to undergraduate veterinary students.

Further case studies and examples will be presented.

EDU-022-005

Training clinicians to make the most efficient use of new data: an evidence-based Bayesian approach

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Objectives: Evidence-based veterinary practice relies heavily on the abilities of clinicians to update their current clinical approaches logically when presented with new evidence. However, the psychological, behavioural and economic literatures suggest that people may react negatively (cognitively, behaviourally, emotionally) when presented with new data.

Aims: (i) to demonstrate a simple practical method for capturing vets clinical beliefs as probability distributions, pre- and post- exposure to new data (ii) to quantitatively assess how logically vets update their beliefs by comparing them to a gold standard: predictions from Bayes theorem

Materials and Methods: In total, 40 vets were interviewed for 30 minutes, face-to-face. Voluntary consent was obtained. A standard script was used and piloted on 3 vets; their data is not included. The clinical context was over-conditioned heifers (body condition score > 3.75) calving down at ≥ 27 months old. The elicited parameter, θ , was the probability of dystocia for this population of heifers when receiving a ruminal bolus of monensin 28 days pre-calving to prevent ketosis, conditional on a 0.5 probability of dystocia without monensin. After a short training exercise, vets expressed their current beliefs for θ as a probability distribution (prior belief) using a version of the roulette method; they placed 20 chips, each worth 0.05 probability in 0.05 discrete intervals over the range of θ . Next, vets were shown data from a small (n=50) clinical trial that challenged their prior belief: if the prior mode was >0.5, data had a point estimate = 0.34 with 95% confidence interval (CI) of 0.22-0.48; if prior mode <0.5, data = 0.66, CI 0.52 - 0.78. Their updated belief after seeing the data was captured as a probability distribution (first revised belief). Finally, they were shown corroborating data from a large, n=1000, trial: data=0.34, CI 0.32-0.38 or 0.66, CI 0.62 - 0.68). Their updated beliefs were then captured again (second revised belief). A conjugate Bayesian analysis was used to calculate, per vet, the logical belief after seeing the first and second trial data. Statistics reflecting central tendency and dispersion of the distributions quantified how logically vets updated their beliefs.

Results: Prior beliefs: Of the 40 vets, 15 had a prior mode >0.5 and 25 had a prior mode <0.5. There was notable heterogeneity in the prior beliefs, both in terms of central tendency and dispersion of their distributions, indicating diversity in current clinical opinion regarding θ . Many vets had non-overlapping prior 95% Bayesian credible intervals.

Changes in central tendency: After seeing the first study results, only 3 vets centred their beliefs perfectly logically. The majority (30/40=75%) moved their centre of locations closer to the data than was logical i.e with respect to where they centred their distributions, they gave more weight to the evidence from the small study than was logical. The remainder (7/40=18%) failed to move sufficiently close i.e they under-valued the new information. In contrast, after seeing the second trial results, 16 vets (40%) moved their centre of location perfectly logically.

Changes in dispersion: After seeing the first study results, the majority (30/40=75%) had less confident beliefs (larger standard deviations) compared to their prior beliefs, i.e. they became more uncertain after seeing the results of the new study than they were before; this is a major departure from Bayesian logic. In contrast, after seeing the second trial results, vets were notably more logical; 35 (88%) had revised distributions with smaller standard deviations relative to their prior. However, given the strength of the new evidence, the majority still remained more uncertain than was appropriate.

Conclusions: Results suggest that some vets may benefit from additional training to help them update their clinical beliefs appropriately when presented with new data. By reflecting on how they revise their beliefs compared to Bayesian logic, vets can learn how best to weigh the value of new data, in the context of their own prior beliefs. The roulette method is a simple practical way to quantify vets' beliefs pre- and post-exposure to new data and could be developed into an on-line training tool. This type of training would be of value to both undergraduates and postgraduates, and will help facilitate the efficient transfer of new evidence into practice.

ED-026-001

Prevalence And Distribution Of Exposure To Schmallenberg Virus In Irish Cattle During November 2012 To November 2013

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Objectives: As Ireland was on the margins of the epidemic in Europe it was uncertain as to how Schmallenberg virus would spread, although given the spread of SBV in several European countries in, it was anticipated that SBV would spread north-west wards during the 2013 vector season. The objectives of this study were to determine the level of exposure among the Irish cattle population initially at the end of 2012 and to document the spread of SBV in Ireland over the course of the 2013 vector season.

Materials and Methods: Samples of brain or external foetal fluids from cases of abortion or still birth submitted to the Dept. of the Agriculture's passive veterinary surveillance system. These cases had suspect lesions such as hydranencephaly or arthrogryposis were tested using a real-time reverse transcription rRT-PCR detecting an 88bp fragment of the S3-segment of the SBV genome developed at the Friedrich-Loeffler Institute. Three national serosurveys were conducted to estimate SBV exposure in Irish cattle, using sera collected between November 2012 and November 2013, as part of the national brucellosis eradication programme. At the first, second and third SBV serological surveys, samples were collected from 3,192 cattle from 529 herds, 3,101 samples from 517 herds, and 3,204 samples from 534 herds, respectively. Sera were screened using a commercially available SBV Antibody Kit (Idexx Laboratories). Data were managed and analysed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and ArcGIS 9.1 (ESRI, Redlands, CA, USA).

Results: Between October 2012 and May 10th 2013, SBV was confirmed by qRT-PCR in brain tissues from 49 malformed bovine fetuses in Ireland. These herds were geographically distributed in the south, south east and east of the country. The overall herd seroprevalences in the three surveys varied from 51.4% to 53.3%, and the overall animal level prevalence was also consistent over the course of the three surveys, ranging from 32.2% to 35.8%, both of which were remarkably consistent, with the greatest rates of seroconversion in the south, south east and east. Herd seroprevalence in counties ranged from 0% to 100%, with the counties in the south and southeast having the highest seroprevalence (>50%), the midlands a moderate seroprevalence (10-50%) while the northern and northwestern counties had a low seroprevalence(0-10%).

Conclusions: The areas with the greatest SBV seroprevalence coincided with the area where SBV was detected by PCR in brain tissue of malformed bovine fetuses. Given the inactivity of the virus since 2012, it is likely that the younger age cohort in herds where SBV was previously endemic are now immunologically naive, and would be susceptible if the virus were to be re-introduced into Ireland. Likewise, substantial proportions of animals on the margins of affected areas have been reported as immunologically naive to SBV, and susceptible to infection if the virus was re-introduced into the country.

ED-026-002

Failure to define the aetiology of acute respiratory disease in thirteen of fifteen live dairy cows in Scotland.

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Objectives: To investigate the aetiology of naturally occurring acute respiratory disease in fifteen live dairy cows on different farms by laboratory examination of bronchoalveolar lavage (BAL) samples, nasopharyngeal swabs, faeces and paired serology using standard laboratory techniques.

Materials and Methods: Previously untreated dairy cows presented for veterinary treatment in a first opinion veterinary practice in Scotland showing clinical signs consistent with acute pneumonia and meeting inclusion criteria designed to maximise the probability of selecting acute cases were admitted to the study. Suitable tubing for BAL sample collection became unavailable after case 8. Samples were examined for infection with the 3 respiratory viruses bovine herpes virus 1(BoHV-1) (nasopharyngeal swab) and bovine respiratory syncytial virus (BRSV) and parainfluenza virus 3 (Pi3) (BAL sample in 8 cases and nasopharyngeal swab in 7 cases). Bacteriology and Mycoplasma spp. culture (BAL sample in 8 cases and nasopharyngeal swab in 7 cases) was also performed. Faeces were examined for lungworm larvae. Paired serology was conducted for BoHV-1, BRSV, Pi3, BVDV and Mycoplasma bovis. Standard laboratory techniques were employed. Clinical signs, case history (including vaccination), treatment and response to therapy were also recorded.

Diagnostic criteria: For the respiratory pathogens (BVDV excluded) demonstration of a pathogen in a BAL sample or nasopharyngeal swab by polymerase chain reaction or virus isolation for viruses or culture for bacteriology or Mycoplasma spp. and detection of lungworm larvae in faeces by Baermann technique. Sero-conversion to the viruses or Mycoplasma bovis would provide supportive evidence provided there had been no previous vaccination with the pathogen in question within 6-weeks of acute sample collection.

Results: Fifteen cases meeting the case inclusion criteria from 10 different farms were sampled between April 2013 and June 2015. Rectal temperature range was 39.3 to 41.4 °C (mean 40; median 39.8; interquartile range 0.55). All cases showed a clear nasal discharge, tachypnoea and harsh respiratory sounds. In 7 cases adventitious lungs sounds were present and in 2 coughing was recorded. No ocular discharge was recorded. Cases 6 and 8 also showed metritis; otherwise no concurrent disease was detected. All cows were Holstein/Friesian, none were in lactation 1 and 3 were in lactation 2 whilst the remainder were in lactation 3 or more. Case 5 was euthanased before an acute blood sample could be collected due to poor treatment response. Of the remaining 14 cases, 2 (cases 6 and 14) had responded poorly to treatment when the convalescent sample was collected whilst the remainder had significantly improved clinically. Diagnosis of a respiratory pathogen was made in only 2 of the 15 cases sampled. Mannheimia granulomatis (historically this would have been identified as Mannheimia haemolytica) in case 3 and Mannheimia haemolytica in case 6 were recovered from BAL samples and case 6 also seroconverted to BVDV. Case 14 seroconverted to BoHV-1 but the virus was not detected. The other 2 seroconversions (to BoHV-1 and BVD) were associated with vaccination.

Conclusions: The failure to confirm a diagnosis in 13 of 15 live cases of acute pneumonia is of interest since the case inclusion criteria were designed to sample acutely infected cattle where any primary pathogen(s) should have been detectable or caused seroconversion.

The failure to culture *Trueperella pyogenes* from any case tends to confirm that chronic cases were not selected.

Comments: The two diagnoses of *M. granulomatis* and *M. haemolytica* are consistent with previous reports of pneumonia in dairy cows investigated at necropsy in the United Kingdom. However, the cases reported here were investigated when they were alive and 12 of 15 responded to treatment and survived so they may represent a different category of diseased animals. The pneumonia in case six may have been secondary to immune suppression caused by BVD virus infection.

The failure to achieve a laboratory diagnosis in 13 of 15 cases challenges current standard laboratory procedures. Maybe the laboratory tests used were insufficiently sensitive or the aetiology was not infectious. Alternatively, the clinical diagnoses may have been incorrect. However, most of the cows responded to treatment for respiratory disease with macrolide antibacterials suggesting that this was unlikely. Most likely, the acute pneumonia was caused by pathogens which are either unknown or not currently routinely tested for and therefore not recognised as significant. It is suggested that further investigation of acute respiratory disease in dairy cows is required to include a broader range of potential respiratory pathogens including unknown and known potential pathogens such as coronavirus and influenza virus.

ED-026-003

Epidemiological Analysis Of A Novel Disease Associated With A New Variety Of Swede In Southern New Zealand

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Objectives: During the winter of 2014 in Southern New Zealand, an unusual pattern of disease and mortality appeared amongst dairy cows grazing on, or with previous exposure to, brassica spp.

The winter of 2014 saw a novel type of swede widely introduced to the market ('HTTM'), and the clinical picture suggested a link between this swede and increased disease and mortality.

This study was a broad epidemiological study that attempted to gather together clinical case series, biochemical analyses, and proportional mortality data to ascertain any association between the novel swede and both disease and mortality.

Materials and Methods: Case series information gathered during this emerging disease included autopsy data from acute deaths, alongside clinical disease and associated biochemical and haematological analyses.

Furthermore, cohort biochemical analyses were performed on adult cows grazing adjacent but different brassica spp; and temporally controlled serological analysis was performed on a group of young heifers (12 months old) assessed prior to, during, and after exposure to the postulated risk factor (the novel swede variety).

Biochemical analysis of peri-parturient (pre partum, colostrum, and milking) exposed and non-exposed cows was also undertaken from a number of affected herds.

Finally, a retrospective analysis was undertaken to measure mortality rates from a large number of herds and cows grazing on a variety of winter grazing options. Farmers were interviewed to gather winter

grazing data, including the type of crop, the day or arrival and of leaving, and the numbers of animals arriving or leaving.

These objective data provided simple mortality rates for separate groups of cows grazing on different forages. Exposure was calculated and a mortality rate (number of deaths/100 cows/100 days) was calculated for each forage.

Relative Risk (RR) and Odds Ratio (OR) were calculated. Then, a Generalised Estimating Equation (GEE) was used, with crop type nested within farm.

The outcome variable was mortality rate (deaths/100cows/100 days), as a linear scale function; and the risk factor was crop type. The model used an independent working correlation matrix and estimated marginal means (EMM) were calculated

Results: The clinical picture was of cows developing a wide variety of symptoms, including inappetence, photosensitivity, non-specific disease and in some cases non-responsive recumbancy. Autopsies typically showed gross liver pathology with histopathological evidence of peri-biliary pathology.

Biochemical analysis amongst clinically affected animals demonstrated vastly elevated liver enzymes (GGT, GLDH, AST); analysis of exposed and unexposed cows showed differences in the same enzymes; and analysis of youngstock showed significant elevations in GGT and GLDH once exposed to HTTM swedes.

When compared with all other winter grazing options, the OR of death on HTTM swedes was 2.29; and the RR of death was 2.28. When HTTM swedes were compared with only other brassica spp (including an HTTM mix), the OR of death on HTTM swedes was 6.15; and the RR of death was 6.11.

Winter crop type had a significant effect on mortality rate ($p < 0.001$). The overall mean MR was 0.64% (95% CI 0.62-0.87). Amongst the key brassica and winter crop types, HTTM swedes had a mortality rate of 2.04% (95% CI 1.64- 2.44); kale was 0.25% (95% CI 0.20- 0.31); and fodder beet was 0.25% (95%CI -0.04-0.55).

Conclusions: These data corroborate the initial clinical hypothesis that there was a significantly higher risk of mortality among adult dairy cows grazing on HTTM swedes during the winter of 2014 than among cows on any other type of forage, irrespective of management or cause of death.

The biochemical, clinical and autopsy data help to confirm that cows developed significant liver disease when grazing the novel swede variety which lead to severe clinical disease and an increased risk of mortality.

These data have helped inform industry advice on the best practice use of this novel swede variety.

ED-026-004

Bovine respiratory pathogens in France: prevalence and new virus discovering

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Objectives: Bovine respiratory disease (BRD) is among the most prevalent pathological conditions in young cattle involving complex interactions between pathogens (virus, bacteria) and environmental or farm management factors. The exact prevalence of each pathogen

and the frequency of co-infection in the different production systems are however still unclear. The objectives of the present study were to use new technologies, next generation sequencing (NGS) and real time PCR (qPCR) or reverse transcription-PCR (RT-qPCR), to best characterize the prevalence of respiratory pathogens in French breeder systems and to identify new respiratory viruses.

Materials and Methods: Nasal swabs (NS) and bronchoalveolar lavages (BAL) were collected from a total of 21 breeding herds with BRD between 2013 and 2015. Four to five calves per herd, not treated with antibiotics, were sampled at the acute phase of infection (< 3 days). After RNA and DNA extraction, BAL and NS samples were tested by real time PCR (Taqvet respiratory pathogens, Lifetechnologies) for bovine respiratory syncytial virus (BRSV), parainfluenza 3 virus, bovine coronavirus (bCoV), bovine viral diarrhoea virus (BVDV), *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni* and *Mycoplasma bovis*. These samples were also screened by a systemic NGS approach using Illumina Miseq technology. According to results, field respiratory specimens (NS, trans-tracheal aspiration, BAL or lung tissue) obtained between 2010 to 2015 from 134 acute respiratory outbreaks in young cattle were tested by real-time reverse transcription-PCR (RT-qPCR) for influenza D virus (IDV) as previously described (Hause et al., PLoS pathogens. 2013;9(2):e1003176). One specimen with the lowest cycle threshold (CT value) was selected for further molecular characterization and its full genome was amplified by PCR and sequenced on a 3130XL Applied Biosystems capillary sequencer. Determination of IDV seroprevalence was done by hemagglutination inhibition assay.

Results: Results on BAL showed a high frequency of co-infections with 2 to 4 pathogens identified in more than 40% of the outbreaks. No specific associations were found; co-infections involved bacteria and virus or virus-virus or bacteria-bacteria. BRSV and BCoV were the most frequently viruses detected in the lower respiratory tract, alone or in association (27% and 42%, respectively). The included pathogens were more frequently detected in upper respiratory tract except for BRSV and *M. bovis* with respectively 26.3% vs 5.3% and 10.5% vs 5.3% frequency in BAL vs NS. Finally 2 of 21 outbreaks remained negative for all tested pathogens. NGS results showed the presence of new viruses in each type of samples. Particularly a new putative respiratory pathogen, called Influenza D virus (IDV) was identified by RT-qPCR in 6 samples (4.5%) out of 134 French field isolates collected between 2010-2014, with CT values ranging from 15 to 35. Co-infections were detected with major pathogens in 4 of the IDV positive specimens. However two samples collected in 2014 were negative for all tested respiratory pathogens, despite reports of clinical signs in the animals. A preliminary serological study (200 bovine sera tested in the region where IDV was detected) indicated a high seroprevalence, with 83% of positive samples. Finally the French IDV complete genome was 94-99 % identical to its American and Chinese counterparts, suggesting a common origin of these new influenza viruses. The estimated ranges of evolutionary distances between French and United States IDV ranged between 0.8 and 5.7%.

Conclusions: French cattle are frequently co-infected with respiratory pathogens although no clear pattern of specific associations was found. Conversely, absence of pathogen detection in respiratory samples suggests that some unknown agents, especially viruses, may be involved in BRD. Recent studies proposed bovine nidovirus, rhinovirus type A or adenovirus type 3 as putative respiratory pathogens. Here we have identified a novel influenza D virus in cattle with respiratory symptoms. Further studies are tough warranted to assess the emergence threat of IDV viruses in Europe, and at providing essential information about the pathogenicity of IDV in cattle.

ED-026-005

Bovine Besnoitiosis: The First Diagnosis of this Emerging Disease in the Republic of Ireland

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Objectives: Bovine besnoitiosis, caused by *Besnoitia besnoiti*, was diagnosed in a dairy herd in Co. Tipperary, Republic of Ireland. This is the first diagnosis of this disease in Ireland or the UK. The objective of this abstract, and the associated presentation, is to document the discovery of this disease in the Republic of Ireland and to reinforce the classification of bovine besnoitiosis as an emerging disease in Europe (EFSA 2010).

Materials and Methods: The diagnosis of besnoitiosis occurred following an investigation on June 19th 2015 into a problem of suspect photosensitisation occurring on a dairy farm since 2010. A number of cows in the herd had clinical signs including thickened skin, alopecia, weight loss and poor milk production. Other cases were described as having clinical signs including pyrexia, swelling of the limbs, respiratory disease and milk drop syndrome which persisted for a period of 2 to 3 weeks. A clinical and epidemiological investigation was carried out on the farm which included taking skin biopsies from severely affected cows.

Results: Skin biopsies carried out on five cows were confirmed to have granulomatous and eosinophilic dermatitis with characteristic large intradermal protozoal cysts consistent with a diagnosis of cutaneous besnoitiosis caused by *Besnoitia besnoiti*. A follow-up investigation was carried out, in conjunction with the Department of Agriculture, Food and the Marine, which involved serological testing and clinical examination of 228 animals on the farm. Serological testing, carried out in the Friedrich Loeffler Institute in Germany, found that 68% (144/212) of animals were ELISA antibody positive for *Besnoitia besnoiti*. In addition, 51% (117/228) of animals had characteristic cysts on the scleral/bulbar conjunctiva (sclerocysts). The prevalence of vulval cysts was 68% (134/198), but there was poor reproducibility and agreement between individual examiners in terms of the identification of vulval cysts. This contrasted with the high level of agreement and reproducibility of results when assessing eyes for the presence of sclerocysts.

Conclusions: The clinical and serological results confirm that this herd is endemically infected with *Besnoitia besnoiti*. It is likely that cases of besnoitiosis have been occurring on this farm since at least 2010. Further epidemiological investigation is on-going to identify the original source of besnoitiosis for this herd and when the disease entered the herd. The identification of this previously exotic disease highlights the importance of disease surveillance at the national and local practitioner level, particularly with respect to emerging diseases in Europe.

Comments: EFSA (2010) Bovine Besnoitiosis: An emerging disease in Europe. European Food Safety Authority Journal 2010 8, 15

EP-034-001

Spatial Differences in the Prevalence of Diseases in a Veterinary Practise

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Objectives: To our knowledge, no analysis has been performed for the catchment area of a single veterinary practice so far. At this local scale, spatial data on disease prevalence can be collected, processed, analysed, and visualised to detect meaningful patterns and validate potential etiologies. In our study, we used a Geographic Information System (ArcGIS®) to represent the spatial distribution of specific diseases among the cattle located in the catchment area of the ambulance servicing the animal hospital of Zurich, Switzerland. In doing this we also assessed linkages between disease prevalence and potential geographical risk factors.

Materials and Methods: We conducted a retrospective study on the prevalence of specific cattle diseases within the catchment area of the ambulance servicing the animal hospital of Zurich. The study window spans between the 1.1.2013 and 31.12.2013, and data has been acquired through the software managing the veterinary hospital services (Oblon®). The cattle at-risk has been estimated based on the records stored in the Swiss animal movement database (TVD) from 1.6.2013. For our study purposes, we used geographical data issued from the Swiss federal office of topography (swisstopo) in 2014, such as background maps, water bodies, road networks, land uses, and altitudes. We defined the individual diseases based on the diagnoses performed at the animal hospital of Zurich and then performed a Kriging analysis to produce prevalence-density surfaces for each disease. The following diseases have been examined: metritis/endometritis, papillomatosis, prolapse uteri, puerperal disease, reproduction problems, retentio secundinarum, torsio uteri, endoparasitosis, foreign body disease, hypocalcemia, ketosis, claw disease, coccidiosis, cryptosporidiosis, and mastitis. Additionally, we evaluated the impact of specific geographical risk factors such as proximity to water bodies, major roads, and altitude.

Results: This study aims at assessing the spatial distribution of the prevalence of specific cattle diseases in the catchment area of a single veterinary practice. The study region is located within the catchment area of the ambulance servicing the animal hospital of Zurich in 2013, which consists of 124 farming companies rising between 20 and 50 cows. The surface investigated is of about 60 square-kilometers and it is located north of the city of Zurich. We performed density surface, cluster and case-control analyses of multiple thematic layers (i.e., disease types and geographic risk factors). Preliminary results suggest that certain parasitoids, especially fasciolosis, could be associated with proximity to water bodies, i.e. rivers, lakes and ponds. Mastitis could also be related to the proximity of wood storage places and forests. Claw disorders could be associated with increasing altitude. However, these findings have to be further validated and bias and confounders should be investigated in depth.

Conclusions: Small-scale risk maps are a useful tool to detect meaningful patterns and validate potential etiologies as well as implementing preventive measures. The implementation of preventive measures associated with the geographical context would reduce the resources employed for cattle diseases treatment, by limiting the costs associated with antibiotic resistance without affecting the cattle health.

EP-034-002

Genetic diversity and antibiotic resistance of E. coli isolates from NCD cases in Uruguay

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Objectives: Neonatal calf diarrhoea (NCD) is a severe infectious disease that affects neonatal calves in Uruguay. High morbidity and mortality rates and the associated economic burden are substantial all over the world. *Escherichia coli* is one of the aetiological agents of NCD, mainly affecting calves in the first hours of life. Genotypic characterization and antibiotic resistance patterns of *E. coli* strains are needed for epidemiological knowledge of NCD.

The aim of this study was to evaluate the genetic diversity, clonal distribution and antibiotic susceptibility patterns of a collection of *E. coli* isolates recovered from calves in Uruguay.

Materials and Methods: A collection of 304 *E. coli* isolates from calves' faeces younger than 35 days-old in Uruguay were analyzed. The collection, generated between 2012 and 2014, included *E. coli* strains recovered from animals affected with NCD and healthy animals at the moment of sampling.

Clonal diversity was analyzed by rep-PCR and multilocus sequence typing (MLST). All isolates were typed using ERIC-PCR whereas 22 *E. coli* strains were selected for multilocus sequence typing (MLST). Selection criteria of strains included previous PCR assays performed to determine the presence of adhesins and toxins genes, presence and absence of symptoms of NCD and geographical origin of the animals.

Dendograms were constructed to establish clonal relationships between ERIC-PCR band patterns by the neighbor-joining method (tolerance 1%, optimization 1%). MLST was performed following the guidelines in the MLST database (<http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/>) and concatenated sequences of the 7 genes of each strain were used to construct a dendogram by the neighbor-joining method.

For selected isolates, the presence of class 1 integrons (C1I) was determined by PCR using I5'-I3' primers. The variable regions (VR) were elucidated by PCR and sequencing with primers 5CS and 3CS.

Resistance to fluoroquinolones (FQ) and oxyiminocephalosporins were evaluated by the ability to grow in Müller Hinton agar plates supplemented with Ciprofloxacin (0.125 µg/ml) and Ceftriaxone (1.0 µg/ml) respectively. *E. coli* ATCC 25922 was used as control. Results interpretation was completed after 24 h of incubation at 37 °C.

Results: ERIC-PCR analyses showed a high genetic heterogeneity. We observed that different genetic variants were present within farm-outbreaks. Also, the same genetic variants were observed in animals with symptoms and without symptoms of the disease. Moreover, it was observed that same genetic variants were circulating in severe outbreaks from neighbor farms. Seventeen different circulating ST were identified, including 4 novel ST. All detected ST were reported from bovine samples, as well as other origins except for ST1087 which was exclusively reported in bovine samples. Particularly, same clone and ST was found in 2 isolates from a single farm. These isolates were from an animal with symptoms of NCD and another animal without symptoms of the disease.

C11 were detected in 5/22 *E. coli* isolates and the variable region showed the following gene cassette arrangement: *dfrA7*; *dfrA17-aadA5*; *dfrA1-aadA1* and *dfrA12-orfA-aadA2*.

Concerning antibiotics resistance, 43.8 % of the 304 *E. coli* isolates were resistant to Ciprofloxacin and 6.3 % to Ceftriaxone; 2.0 % were resistant to both Ceftriaxone and Ciprofloxacin.

Conclusions: Several *E. coli* genetic variants with diarrhoeagenic potentiality for cattle were detected in Uruguay. In total, 17 ST were identified, including 4 novel ST. ST29 and ST69 complexes, widely distributed in bovine and human were detected.

Diversity results demonstrated that factors like the status of the animal, environmental conditions and occurrence of others pathogens individually or together, could have a role in NCD development.

Finally, a large number of strains were resistant to FQ and the presence of C11 explain resistance to co-trimoxazole and aminoglycosides confirming that antibiotic resistance is an increasing problem in livestock.

EP-034-003

A systematic review and meta-analysis of clinical mastitis recurrence in dairy cows

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Objectives: A high proportion of cows with a clinical mastitis (CM) case will experience repeated CM events within the same lactation. It is not clear, however, whether CM risk following a first CM event is decreased (i.e. efficient immunity), maintained, or increased (i.e. greater susceptibility). Objective of the current study was to compare risk of subsequent CM in cows that already experienced a first CM in the current lactation to risk of CM in healthy cows.

Materials and Methods: The study design was a systematic review and meta-analysis of the literature. Three databases were searched for original studies describing CM recurrence. Articles focusing strictly on first parity cows and articles not following cows for their complete lactation were excluded. In each article, data regarding total number of lactations followed and number of lactations with ≥ 1 and ≥ 2 CM cases were extracted. The source of data used in each study (i.e. prospective data collected specifically for the study vs. use of an existing database) and CM recurrence case definition (i.e. the minimal number of days between two episodes of CM to consider them independent cases) were also extracted. For each study, a risk ratio (RR) measure comparing risk of subsequent CM cases in cows having already experienced a first case in the current lactation to that of healthy cows was computed with 95% confidence intervals (CI). Summary RR across studies were computed using random effect models. Putative sources of heterogeneity were investigated using subgroup analyses and univariate meta-regression.

Results: A total of 1,666 abstracts were reviewed; nine studies were included in the meta-analysis (see references). In three studies, CM

data were collected from existing databases. Three studies used ≤ 24 h between cases as minimal lag time between events and six studies used values ranging from five to 21 days. Individual studies RR ranged from 0.83 to 2.1. Important heterogeneity between studies was noted. Results from the univariate meta-regression indicated that source of CM data used explained only 3.9% of the between studies RR variance. The minimal lag time between two independent CM cases (categorized as ≤ 24 h vs. ≥ 5 days), however, explained a substantial proportion (62.2%) of the between studies RR variance. Summary RR of 1.5 (95% CI: 1.2, 2.0) and of 0.99 (95% CI: 0.86, 1.1) could be computed for studies using minimal lag times of ≤ 24 h, and ≥ 5 days, respectively.

Conclusions: Our results suggest that cows are, at the very least, equally susceptible to CM after a first case. Clinical mastitis case definition, however, appears to substantially affect the computed RR. Most important heterogeneity was noted when comparing studies using ≤ 24 h to that using ≥ 5 days between cases.

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Evolution of the nasopharyngeal microbiota of beef cattle from weaning to 40 days after arrival at a feedlot

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Objectives: Bovine respiratory disease (BRD) is a major cause of morbidity and mortality in beef cattle. Whereas host immunity is important in controlling overgrowth of bacterial pathogens in the nasopharynx, there is recent evidence suggesting that the nasopharyngeal microbiota has a key role in respiratory health. However, there is a paucity of knowledge regarding evolution of nasopharyngeal microbiota when cattle are most likely to develop BRD; from weaning to 40 d after arrival at a feedlot.

The objective was to describe the composition of the nasopharyngeal microbiota of beef cattle and its evolution from weaning to 40 d after arrival at a feedlot.

Materials and Methods: Deep nasal swabs (DNS) from 30 Angus-cross steers were collected at weaning, on arrival at a feedlot, and at 40 days after arrival. The DNA was extracted from DNS and the hypervariable region V3 of the 16S rRNA gene was amplified. The 16S rRNA gene amplicons were then sequenced using the MiSeq reagent kit v2 (500 cycles) on the MiSeq platform (Illumina, Inc., San Diego, CA, USA). Sequence data were processed using a custom Snakemake pipeline (<https://bitbucket.org/mlworken/microbiome-pipeline>) based on the UPARSE pipeline using USEARCH v8.0.1623. Downstream analysis was performed in R (R Core Team 2015, Vienna, Austria) using functions from phyloseq, DESeq2, and vegan. Intra-sample diversity (α -diversity) was calculated using observed species and the Shannon diversity index; α -diversity between groups was compared with a Kruskal-Wallis Rank Sum test (using $p < 0.05$ as a cutoff for significance). The OTUs that changed over time were identified using a generalized linear model as implemented in DESeq2.

Results: A total of 10,494,168 sequences were obtained across all samples, with a total length of 175 bases per read post-processing, and an average coverage of 111,640 sequences per sample (range = 2,911-314,407). Sequences were clustered into 1342 OTUs (excluding singletons) across all samples.

In total, 16 phyla were identified, although 87.9% of the total abundance belonged to Tenericutes (53.2%) and Proteobacteria (34.7%). The remaining phyla that each comprised $> 1\%$ of OTUs were Firmicutes (4.2%), Bacteroidetes (3.7%) and Actinobacteria (3.4%). Within Tenericutes, 99.8% of the abundance was assigned to a single genus; *Mycoplasma*. BLASTing the most abundant OTUs that were classified within this genus showed that *Mycoplasma dispar* was the most prominent species (78.5 % of the abundance within this genus), followed by *Mycoplasma bovirhinis* (4.5%). The Proteobacteria phylum was comprised mostly of *Pasteurella* (32.4%), *Ralstonia* (29.5%), *Moraxella* (26.2%) and a smaller percentage of *Mannheimia* (2.3%).

A distinct shift in the community profile was apparent among time points (i.e. weaning, on arrival at a feedlot, and 40 d after arrival). In total, 92 OTUs changed over time, as identified using a generalized linear model approach. With regards to α -diversity measures, there was no difference among time points in the number of observed OTUs ($p = 0.87$) and Shannon diversity ($p = 0.10$).

Conclusions: (i) The nasopharyngeal microbiota of cattle underwent a rapid and profound evolution from weaning to arrival at the feedlot and during the first 40 days at the feedlot; and (ii) *M. dispar* and *M. bovirhinis* dominated the nasopharyngeal microbiota of healthy cattle. Because an evolving bacterial community may be less capable of resisting colonization by pathogenic bacteria, the instability of the nasopharyngeal microbiota documented in this study might explain why cattle are most likely to be affected with BRDc during the first weeks after weaning.

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Effectiveness of collective treatments in the control of bovine digital dermatitis. Where is the evidence?

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Objectives: Formaldehyde and CuSO₄ have been used in footbaths as the standard treatments of bovine digital dermatitis (BDD). Others several collective treatments (CT) are commercialized, frequently without evidence of their effectiveness. The objective of this exhaustive review was to scrutinize the clinical evidence that support collective treatments in the control of BDD. For each study, preventive and curative outcomes were expressed through numbers needed to treat (NNT) an original, easy and useful measure of the treatment benefit, which represents the number of animals that must be treated to make a positive or negative difference on one animal.

Materials and Methods: Clinical evidence was collected across an evidentiary search strategy that included electronic data-bases, 375 citations were screened for relevance by the authors and 15 references were selected. Additionally, a manual search of the grey literature was performed, and 8 additional relevant reports were identified. Altogether, 23 relevant reports were included to be verified by the authors through a screening tool specifically designed for this review. Finally 15 studies were included, excluding trials with missing information and limiting the evidence to (i) trials conducted in dairy cattle herds (ii) where the disease occurred naturally and (iii) that compared CT to a negative control or placebo group. Two outcomes were differentiated. The therapeutic outcome was defined as the positive effect measured by reduction on the persistence and recurrence of BDD lesions. The preventive outcome was defined as the positive effect of the intervention to limit the occurrence of a new BDD lesion. First, the outcomes were summarized and the absolute risk reduction (ARR) was calculated by the difference in percentage of clinical success between the treated and the negative control or placebo group. The NNT statistics were obtained by dividing, the ARR into 100%. The small number of studies included was due mostly to the absence of comparative negative controls in the study protocols, the missing information and the absence of significant results (5 of 15).

Results: For the curative outcome, the few treatments that were reported as significantly effective ($P < 0,05$) were, (i) Oxytetracycline (25mg/ml) sprayed 8 times in hoofs prerinsed with water once daily, 5 consecutive days, no treated for 2 days, and then treated 3 times every other day (NNT=2); (ii) Commercial formulation of soluble copper, peroxide compound, and a cationic agent sprayed 8 times in hoofs prerinsed with water once daily, 5 consecutive days, no treated 2 days, and then treated 3 times every other day (NNT=2); (iii) Acidified ionized copper (0.6%) in footbaths administered 5 times, twice daily for periods of 10 days aprox. the product was renewed each 300 passages (NNT=3); (iv) Water prerinse footbath+CuSO₄ pentahydrate (25%) in footbaths administered after 4 consecutive milkings every week during 5 weeks, the product was renewed each 200 passages (NNT=4); (v) Foam tensides and peracetic acid system administered twice a day on alternate weeks during 8 weeks (NNT=5); (vi) Tap water mixed with soap solution (0.4%) administered through an automatic hoof washer twice a day during 8 weeks (NNT=12).

For the preventive outcome the treatments that were reported as significantly effective ($P < 0,05$) were (i) Cooper and zinc chelates (50%) sprayed in hoofs prerinsed with water after 2 milkings 4 days apart every 2 weeks during 6 months (NNT=15); (ii) Cooper and zinc chelates (5%) in footbaths administered to hoofs prerinsed with water after 4 consecutive

milking every 2 weeks during 6 months, the product was renewed each 150 passages (NNT=25).

Conclusions: As a consequence, this review reveals that there is weak scientific evidence supporting the preventive or therapeutic effectiveness of collective treatments against BDD, explained mostly by the high particularities of the treatment protocols and populations that make difficult extrapolate the results to other populations. These findings support the importance to develop uniform protocols including negative controls for trials evaluating the effectiveness of collective treatments.

Comments: The NNT can only be compared within the same trial because it represent the effect of a treatment compared to a control or placebo group under the specific conditions of the study, indicating the spontaneous cure rate and the severity of the disease challenge into a specific diseased population.

The comparison of the collective treatments effectiveness on the control of BDD is complicated by multiple causes. Firstly, a high recurrence of the disease after an initial clinical resolution, and a variable rate of spontaneous cure, even up to 60%. Finally, there are important differences across populations and trials protocols and also in terms of NNT animals among effective treatments (varying from 2 to 25).

GB-047-001

Prevalence and Discovery of Genetic Defects in the National Irish Cattle Population

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Objectives: Genetic diseases occur in every cattle breed worldwide. Genetically superior animals can be carriers for known genetic defects; therefore, screening and strategic mating is the best method to control disease frequency. Methods to identify genes of interest in animals where a causative mutation has not been found must be utilized to maximize the genetic potential of cattle. This study endeavoured to do both in the Irish national cattle population using genotyping with the International Dairy and Beef (IDB) SNP chip to determine carrier status for 38 mutations and through exome sequencing to identify punitive mutations for progressive ataxia.

Materials and Methods: Genetic Disease Frequency: DNA from independently confirmed carrier or affected animals was used to validate the diagnostic ability of 65 disease and trait SNP probes on the International Dairy and Beef (IDB) SNP chip. Since 2013, 170,992 Irish animals have been IDB genotyped and allele frequencies from the validated disease probes were analysed to calculate carrier frequencies for 38 mutations that are causative for 33 different diseases. Some genes have multiple mutations that result in the same disease phenotype. This provides a snapshot of the prevalence of the 38 disease-causing alleles across multiple breeds, for pedigree and commercial animals, and for AI and stock bulls.

Genetic Disease Causative Mutation Discovery: A genetic disease in Charolais cattle, Progressive Ataxia, was farmer identified, veterinarian confirmed, and reported to ICBF. Pedigree analysis of affected animals supported an autosomal recessive disease model. Tissue from two affected paternal half-sib animals and their parents were collected, DNA was extracted, and IDB genotyped. Genotypes were analysed using SNP & Variation Suite v8x (Golden Helix, Inc., Bozeman, MT) to identify extended runs of homozygosity (ROH) that were unique to the affected animals. DNA from affected animals was sent to CoFactor Genomics for Exome sequencing. Exome sequence variants were compared to a reference population of 244 whole genome sequenced *Bos taurus* individuals to identify variants that were unique and homozygous in the affected animals and were within the ROH.

Results: Disease Mutation Allele Frequencies: Of the 38 disease-causing mutations, 27 are segregating in the Irish national beef and dairy herd. While the national dairy herd is primarily Holstein-Friesian the beef herd is a composite of multiple breeds, purebreds, and crossbred animals. Typically, the frequency of a disease mutation is determined via genotype data from the pedigree and AI population. This study's data set provides a rare insight into allele frequency differences between a country's commercial and pedigree herds. Most of the disease-causing alleles are at a higher frequency in the commercial than the pedigree herd, and are also higher in frequency in stock than AI bulls. Of concern, some of the mutations that require payment of a separate royalty fee for genotyping including: Brachyspina, Complex Vertebral Malformation, and Tibial Hemimelia-Improver have carrier rates >3% within at least one breed.

Progressive Ataxia Putative Causative Mutations: Through Exome sequence analysis 8 SNP were identified as candidate causative

mutations, however, through further examination of the literature it has been deemed possible that the causal mutation may lie in a genomic region that is not covered by exome sequencing. These SNP were included in the design of the IDBV3 and their allele frequencies in the national herd and genotype status of affected animals will be used to identify if any of the exome-sequencing detected SNP are the causal mutation.

Conclusions: Through the identification of carrier animals in a population, better mating strategies can be implemented to reduce the chance of producing animals affected by genetic diseases when causative mutations are known. Ireland began this process with the development of the IDB chip and will continue to add new disease-causing SNPs as they are published. While exome sequencing produced 8 candidate casual mutations for progressive ataxia, the data will be strengthened by whole genome sequencing of the affected animals, and future validation studies. This method will then be used to identify the variants causing new genetic diseases as they appear.

GB-047-002

Estimation of genetic parameters of postpartum health disorders in Holstein cows

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Objectives: The objective was to estimate the genetic parameters of: a) serum Ca, Mg, P, K and β -hydroxybutyric acid (BHBA) concentrations; b) the associated subclinical macromineral disorders, and c) clinical diseases of dairy cows during the first 8 days after calving.

Materials and Methods: The study included 1,021 Holstein dairy cows (1 - 4+ lactations) in 9 herds. No herd used preventive measures for hypocalcemia. Clinical examination and blood sampling was performed on DIM 1, 2, 4 and 8. Clinical cases of milk fever (MF), retained fetal membranes (RFM), metritis (MET), mastitis (MAST), left/right abomasal displacement (LDA/RDA), ketosis (KET), and uterine prolapse (UP) were recorded on the same days. Serum concentrations of Ca and Mg were determined with AAS, while P and K with biochemical and electrolyte analyzers. BHBA was measured with a spectrophotometer in samples of day 8. The final data set included 4,020 Ca, 4,019 P, 4,020 Mg, 3,792 K, 997 BHBA measurements and 4,064 clinical records. All pedigree available was included in the analysis bringing the total number of animals to 4,262. Macromineral-related traits defined as: subclinical hypocalcemia (SCHCa): Ca \leq 8.3 mg/dL; hypophosphatemia (HypoP): P \leq 4.2 mg/dL; subclinical hypomagnesemia (SCHMg): Mg \leq 1.8 mg/dL; hypokalemia (HypoK): K \leq 3.9 mEq/L; and hyperphosphatemia (HyperP): P \geq 7.80 mg/dL. Macromineral and clinical disease-related traits were analyzed with a univariate random regression model. A similar single record model was used for the analysis of BHBA concentration. Genetic correlations among all the above traits were also estimated with a series of bivariate analyses. The genetic analysis for Ca, Mg, P and K and the associated health traits included 986 cows, which showed no obvious signs of MF (35 cows with MF were excluded). The analysis for the other health traits included all 1,021 cows.

Results: Mean serum Ca, P, Mg K and BHBA concentration (\pm s.e.m.) was 8.92 \pm 0.018 mg/dL, 5.21 \pm 0.020 mg/dL, 2.24 \pm 0.006 mg/dL, 4.58 \pm 0.009 mmol/L, 829.03 \pm 602.29 μ mol/L, respectively. Daily heritabilities of Ca

(0.23 - 0.32), P (0.30 - 0.43) and Mg (0.20 - 0.39) were moderate to high, while those of K were low to moderate (0.12 - 0.23). Heritability of BHBA concentration was 0.19±0.09. Daily heritabilities of SCHCa (0.13 - 0.25), HypoP (0.18 - 0.33), SCHMg (0.11 - 0.38) and HyperP (0.14 - 0.22) were low to moderate, while that of HypoK was low (0.08 - 0.10). All these estimates were statistically greater than zero ($P < 0.05$). Statistically significant daily heritabilities ($P < 0.05$) of postpartum health diseases were those of MF (0.07 - 0.11), MAST (0.15 - 0.41) and LDA (0.19 - 0.31). No significant genetic correlations among biochemical traits and clinical or subclinical health traits were found.

Conclusions: Serum concentrations of Ca, P, Mg, K and BHBA, as well as SCHCa, HypoP, HyperP, SCHMg, HypoK, MF, MAST and LDA during the first 8 days after calving are heritable traits in Holstein cows. Genetic selection could contribute to minimize the incidence of these disorders.

GB-047-003

A stop-gain in the laminin, alpha 3 gene causes recessive junctional epidermolysis bullosa in Belgian Bleu cattle

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Objectives: Intensive selection for desired production traits, exacerbated by the extensive use of artificial insemination, drastically reduces the effective population size of livestock populations including cattle. This leads to frequent outbursts of genetic defects, which are causing major economic and welfare concerns. We have established a heredo-surveillance platform in the Belgian Blue Cattle breed (BBCB) highly selected for meat production to (i) monitor the emergence of novel genetic defects, (ii) identify causative loci and mutations by positional cloning, (iii) develop and offer indirect and direct diagnostic tests.

Materials and Methods: Four newborn purebred Belgian Blue calves presenting a severe form of epidermolysis bullosa were recently referred to our heredo-surveillance platform. SNP array genotyping followed by autozygosity mapping located the causative gene in a 8.3-Mb interval on bovine chromosome 24. Combining information from (i) whole-genome sequencing of an affected calf, (ii) transcriptomic data from a panel of tissues and (iii) a list of functionally ranked positional candidates pinpointed a private G to A nucleotide substitution in the Laminin, alpha 3 (LAMA3) gene that creates a premature stop codon (p.Arg2609*) in exon 60, truncating 22% of the corresponding protein.

Results: The LAMA3 gene encodes the alpha 3 subunit of the heterotrimeric laminin-332, a key constituent of the lamina lucida that is part of the skin basement membrane connecting epidermis and dermis layers. Homozygous loss-of-function mutations in this gene are known to cause severe junctional epidermolysis bullosa in human, mice, horse, sheep and dog. Overall, our data strongly support the causality of the identified gene and mutation. We developed a TaqMan-based direct diagnostic test to interrogate the c.7825C>T mutation. The four cases were confirmed homozygous mutant, and a carrier frequency of ~1% was estimated within the BBCB by screening a cohort of 3,000 animals.

Conclusions: Jointly with all previously identified deleterious and functional mutations in BBCB, direct genotyping of this new variant is now routinely applied on a low-density custom array to avoid carrier-carrier matings in breeding schemes. This study confirms the power of state-of-the-art genomic tools to rapidly elucidate the molecular basis of emerging monogenic disorders in livestock.

GB-047-004

Conserved DNA elements may influence expression of reproduction-associated bovine beta-defensins

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Objectives: β -defensins are an important class of host defence peptides (HDPs). A growing body of evidence has implicated these genes in the fertility of several species, particularly a syntenic cluster which is predominantly or solely expressed in the male reproductive tract. Having been the first to confirm the existence of these genes in the bovine genome, we are interested in elucidating their role in reproductive function and fertility. As the mechanisms regulating these pleiotropic molecules are poorly understood, we aimed to bioinformatically analyse this gene region to identify probable regulatory elements and stimuli driving transcription.

Materials and Methods: Our comparative genomics approach centred on the identification of conserved non-coding regions in the β -defensin gene cluster across multiple species. Gene regulation at the transcription level is mediated by transcription factors which participate in chromatin modelling and bind DNA to enhance or repress transcription. These proteins recognise short DNA motifs of specific sequence which can be found in gene-proximal promoter regions or in distal enhancers, meaning such functional DNA elements are highly conserved. 5kb promoter regions from a minimum of 8 mammalian orthologs of each β -defensin gene were analysed using oPOSSUM3 software for transcription factor binding sites which were over-represented compared to a length- and GC-matched background dataset of randomly-selected human gene promoters. 300kb of the β -defensin gene region from 5 species separated by over 80MYA of evolution was aligned using a threaded-blockset approach. Following commonly-used criteria, a DNA sequence conservation cut-off of 70% over 100bp is the minimum required to identify an evolutionarily-conserved region (ECR). A more stringent cut-off of 77% over at least 350bp is thought to be indicative of a core enhancer with increased importance. These DNA regions were analysed using a web-based tool called CLARE which builds a predictive model of features common to the input set versus a sequence-scrambled background set of DNA sequences.

Results: DNA binding sites for several transcription factors are statistically overrepresented in the 5'UTR promoters of β -defensin genes versus a control background gene set. These include androgen receptor, glucocorticoid receptor, retinoic acid receptor, PAX5 and RFX1. These transcription factors are all expressed in the testis and several have been shown to be important in regulating spermatogenesis. The β -defensin gene region contains basic ECRs located in immediate 5' and 3' regions, intronic and intergenic regions. Three putative core enhancers were identified in silico. An enhancer located within a subset of β -defensins solely expressed in the reproductive tract of the sexually mature male contains binding sites for androgen receptor and AP-2 α , a chromatin-

opening transcription factor known to co-operate with androgen receptor in the murine epididymis. A second enhancer contains binding sites for GATA1 and RFX1, which have also been implicated in spermatogenesis.

Conclusions: A comparative genomics approach is a powerful first step toward understanding evolutionarily conserved regulatory mechanisms for these multifunctional genes. The signature of over-represented transcription factors agrees with the observed expression pattern of these genes. Identified intergenic ECRs may act as distal regulatory elements to control expression of one or several male reproductive tract-specific β -defensins in tandem. These regulatory elements will be targets of experimental assays to determine their contribution to transcriptional regulation of β -defensin genes and bovine fertility.

herds with two and three breed animals increased from 1% in 1990 to 17% in 2013. Very few three-breed crossbreeding herds were identified.

Conclusions: Of the farms in Australia which are regularly milk-recording, the most common breed structures are the single breed purebred herd and the "less serious" 2-breed crossbred herd, with both comprising around one-third of herds in recent years. These results show that a substantial proportion of Australian dairy herds contain some crossbreds but few have used cross-breeding on an ongoing systematic herd-wide basis. Further research will describe these breed patterns in more detail. As some herds may benefit from crossbreeding, further studies are required to better understand farmer beliefs about, and experiences of, crossbreeding.

GB-047-005

The use of crossbreeding in Australian dairy herds

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Objectives: The objective of this study was to describe herd breed structures over time for a large subset of Australian dairy herds. The main area of interest for this study was the use of crossbreeding by dairy farmers; specifically proportions of farms using crossbreeding, and whether they were using 2-breed or 3-breed systems.

Materials and Methods: The study was a retrospective descriptive study. Data was from milk-recording herds extracted from the Australian Herd Improvement Scheme (ADHIS) database. The unit of interest was the herd-year. Breed categories for herd-years were defined as: 1) purebred herds: further divided into a) single breed herds: a herd-year where >95% of calvings were to purebred cows, $\leq 1\%$ of the calvings were to F1 cows, and only one purebred had >5% of calvings b) dual breed herds: as above but two purebreds had >5% calvings each, c) purebred herd with more than 2 breeds: as above but more than two purebreds had >5% calvings each, and d) purebred herd containing a few crossbreds: a purebred herd (>95% of calvings were to purebred cows) but >1% of calvings were to F1 cows); 2) two-breed crossbreeding herds: further divided into "more serious" or "less serious": "more serious": >50% of calvings by 2 year-old cows were F1 (e.g. JJFF) or backcross (e.g. FFJF or JJJF): "less serious": >0% and $\leq 50\%$ were F1 or backcross; 3) three-breed crossbreeding herds: >50% of calvings by 2 year-old cows were to a 3-breed backcross and none were to a 2-breed cross; 4) crossbreeding herd with two and three breed animals: (of the calvings by 2 year-old cows in that herd-year, at least 1 was to a 2-breed and at least 1 was to a 3-breed animal); 5) non-purebred herd with all purebred 2 year-olds: a herd in which $\leq 95\%$ of the calvings in the herd were to purebreds, but all 2 year-old cows that calved were purebred.

Results: Within each herd, each calendar year (ie each herd-year) from 1990 to 2013 with sufficient data was categorised. The most common herd breed categories were single breed purebred herds (38% of all herd-years; 33% to 38% of herds in each year from 2010 to 2013) and "less serious" two-breed crossbreeding herds (35% of all herd-years; 33% to 36% of herds in each year from 2010 to 2013). Dual breed purebred herds were rare (1% of all herd-years), as were "serious" two-breed crossbreeding herds, (although the proportion of herds that were this category increased slightly from 1% of herds in 1990 to 5% in 2013). The proportion of herds that were purebred herds containing a few crossbreds fell from 23% in 1990 to 6% by 2013, while crossbreeding

IV-027-001

Associations between immune competence, stress responsiveness and production in Australian Holstein-Friesian heifers

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Objectives: The objectives of this study were to assess and classify adaptive immune responsiveness in first lactation Holstein-Friesian heifers reared under a pasture-based production system and to determine relationships with stress responsiveness, resistance to internal parasites, udder health and production performance. Furthermore, immune responsiveness in purebred Holstein-Friesians and crossbred Holstein-Friesians were investigated.

Materials and Methods: A commercial vaccine was used to induce measurable antibody and cell-mediated immune responses, and animals were classified as High, Medium and Low responders for antibody (AMIR), cell-mediated (CMIR) and combined immune response (CIR). Short-term handling and yarding effects measured stress responsiveness, fecal egg count measured resistance to internal parasites, somatic cell counts assessed udder health and production performance was assessed using estimates of total daily milk volume, milk fat and milk protein. A total of 403 pre-joining heifers from two herds in a seasonally calving, pasture-fed production system were enrolled in the study and followed to the end of their first lactation cycle.

Results: Tests for correlations between stress and immune responses showed a negative correlation with AMIR and a trend towards positive correlation with CMIR ($R=0.068$). The crossbred-bred Holstein-Friesians recorded a higher AMIR compared to the pure bred Holstein-Friesians, and there were no differences in CMIR. Fecal egg counts were generally low and the assessment for resistance to internal parasites showed no significant correlations. Linear individual cow somatic cell scores of 2.39 - 2.68 were recorded within the lactation cycle with significant differences between the early and late lactation period. No significant differences were recorded between the immune response groups in the linear cell counts and milk yield. A negative correlation was observed between milk yield and the magnitude of AMIR whereas a positive correlation was observed with the intensity of CMIR. A significant difference was recorded in fat content in the AMIR group with high responders recording higher 305 day fat yields compared with their low responder counterparts. No significant difference was observed for the CMIR and CIR groups. No significant differences were observed in protein content between the immune response groups.

Conclusions: From this study, it is concluded that stress and AMIR were negatively correlated and immune responses were variable within the breeds. The high-AMIR responders had better milk quality compared with other immune responders and no relationships were observed between SCC and resistance to internal parasites with immune responsiveness.

IV-027-002

Influence Of Maternal Antibodies On Induction Of Vaccine Immune Response Against Respiratory Viruses In Holstein Calves

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Objectives: The interface between passive and active immunity in calves may represent the main risk factor for bronchopneumonia, since the decay of maternal antibodies (Abs) encourage the infection by respiratory viruses. The remaining Abs, even if not protect the newborns, could exert a suppressive effect on the development of humoral immunity induced by vaccination. Thus, the hypothesis is that circulating maternal Abs blocks the vaccinal response. The purpose of this research was to evaluate the influence of maternal Abs on vaccine immune response to viruses involved in Bovine Respiratory Disease (BRD) in calves with 14, 90 and 180 days of age.

Materials and Methods: The newborns received 4L of colostrum in the first 12 hours of life, obtained from colostrum donor cows vaccinated with Cattle Master Gold FP5+L5® (Zoetis, Brazil), composed by inactivated strains of BVDV type 1 (5960) and type 2 (53637); live/thermosensitive BoHV-1 (Cooper) and BPIV-3 (RLB 103); live/attenuated BRSV (375) and Leptospira. The animals were randomly distributed into four groups: G1 - vaccinated at 14d and booster at 44d (n=6); G2 - at 90d and booster at 120d (n=5); G3 - at 180d and booster at 210d (n=8). Control group (CG, n=5) non-vaccinated also was evaluated from birth up to 240 days of life. Blood samples were harvested at vaccination (T0), booster (T1) and 30 days after second dose (T2).

Results: The vaccine did not promote increase in Abs titer (Log₂) for BVDV in any of the groups. Calves from G1 presented titers for BVDV of 12.3 (10.3-12.3) at T0 and 8.8 (7.3-11.3) at T2; the G2 showed titers of 7.3 (4.3-8.3) at T0 and 6.8 (3.3-7.3) at T2, and G3 titers of 5.3 (0.0-6.3) at T0 and 7.3 (0.0-10.3) at T2. Same values could be observed in the control groups at all moments. The Abs titer to BoHV-1 observed in G1 were of 6.5 (6.0-7.0) at T0 and 6.0 (5.0-8.0) at T2; in G2 of 4.5 (0.0-6.0) at T0 and 4.0 (0.0-10.5) at T2 and G3 of 7.5 (0.0-10.0) at T0 and 10.0 (0.0-10.0) at T2. Differences could be demonstrated only between G3-CG3 ($P=0.01$). BRSV presented different profile with Abs titers of 3.5 (0.0-4.0) at T0 and 3.5 (0.0-4.0) at T2 in G1, of 0.0 (0.0-3.0) at T0 and 0.0 (0.0-3.0) at T2 in G2, and of 5.0 (3.0-10.0) at T0 and 8.5 (4.0-10.0) at T2 in G3. Similar values could be found for the CG, except at T0 ($P=0.04$) with high titers for CG3 (0.0-7.0), and at T2 ($P=0.01$) with low titers for CG3 (0.0-4.0). For BPIV-3, the Abs titer for G1 were of 8.0 (7.0-10.0) at T0 and 5.5 (3.0-9.0) at T2, for G2 were of 4.5 (0.0-5.0) at T0 and 5.0 (0.0-5.0) at T2 and for G3 were of 5.0 (0.0-10.0) at T0 and 10.0 (0.0-10.0) at T2. Differences could be detected between CG at T0 ($P=0.02$), demonstrating high titers for CG2 (4.0-7.0), and at T2 ($P=0.05$) with low titers for CG3 (0.0-5.0). No differences among moments were observed ($P<0.0167$) for any of the viruses. The susceptibility for bronchopneumonia could be demonstrated from 90-120d, since 4/5 (80%) calves were sick in non-vaccinated group.

Conclusions: Maternal immunity and vaccine response presented inverse relationship. Thus, the early vaccination in the G1 did not promote increase in Abs titer for any of the viruses, while the vaccination at 90 days of life (G2) and 180d (G3) improved the Abs response after booster due to the decay of maternal Abs at these ages.

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IV-027-003

Switch your energy supplier – and treat less infectious diseases.Dirk Werling ^{1,*}¹Pathology and Pathogen Biology, Royal Veterinary College, Hatfield, United Kingdom

Objectives: One of the truly remarkable discoveries in modern biology is the finding that the nervous system, endocrine system and immune system use a common chemical language for intra- and inter-system communication. This has severe consequences, especially in such times where a situation of high-energy expenditure exists in a host, which becomes aggravated through the fact that the host has to mount an additional immune response

Materials and Methods: Here, I will summarise our current understanding regarding the energy consumption to mount an immune response, and will discuss the potential consequences in light of physiological occurring times of high energy expenditure, especially during the peri-parturient period. Indeed, during this period it may be beneficial to the host to spend energy on prophylaxis, rather than treatment. Here, treatment using immunomodulators, naturally occurring proteins of the immune system, may provide a new way forward.

Results: Indeed, immunomodulators, such as granulocyte-colony stimulating factor have been tried in various experimental approaches for treatment of infectious diseases in humans and farm-animals. However, only recently have these immunomodulators been produced in a form that makes their usage substantially more appropriate for treatment, of certain cancer forms in humans. However, it seems that the use of similar, "own" molecules with a modification that increases their half-life may also aid and/or prevent the occurrence of economically important infectious diseases in animals.

Conclusions: The use of proteins produced by the host itself in a recombinant form may provide a new ways forward to tackle old problems by prevention rather than treatment of infectious diseases in a time when the over-use of antibiotics has led to an increase in multi-drug resistant bacteria as well as an increased consumer awareness of the value of food products.

Such a shift from re-active energy expenditure to a prophylactic, active energy expenditure may prevent energy losses in the long term, leading to a faster restoration to homeostasis and therefore an overall increase in overall animal welfare.

IV-027-004

Passive Immunity In Calves Stimulated By Vaccination Of Dry Cows For SalmonellaGeof Smith ^{1,*}, Derek Foster ¹¹Population Health and Pathobiology, North Carolina State University, Raleigh, United States

Objectives: Salmonella infections are an economically important disease of cattle and also represent a worldwide public health concern. Although cattle of all ages can be infected with Salmonella bacteria, death is most often reported in calves less than 8 weeks of age. The goal of this research was to determine whether vaccinating cows at dry off with commercially available Salmonella vaccines would result in the

presence of Salmonella specific IgG antibodies in the colostrum of cows at calving and whether these colostral antibodies would be transferred to the calf.

Materials and Methods: Two different prospective clinical trials were conducted using 2 different commercially available Salmonella vaccines. In one study, 30 Holstein cattle from a dairy herd naïve to Salmonella were vaccinated at dry-off with a Salmonella enterica serovar Newport bacterin and again 4 weeks later. An additional 30 cows received only saline and served as an unvaccinated control group. In a separate trial 30 Holstein cows were vaccinated 3 weeks before the end of lactation with a Salmonella enterica serovar Dublin vaccine with a second dose given at dry-off. Once again an additional 30 cows received only saline and served as a control group. Calves from both studies received fresh colostrum from their dam within 2 hours of birth, and had blood collected 24 hours later. Serum and colostral antibody titers for S. Newport or S. Dublin antibody concentrations were determined using enzyme-linked immunosorbant assays (ELISA).

Results: Cattle vaccinated with the S. Newport bacterin had elevated S. Newport antibody titers at calving in blood (P = 0.01) and colostrum (P = 0.011). Calves that received colostrum from vaccinated cattle also had significant increases in Salmonella antibody titers (1.04 + 0.03 presented as S/P ratio) as compared to calves born to unvaccinated cows (0.30 + 0.02). Cattle vaccinated with the Salmonella Dublin vaccine had elevated S. Dublin antibody titers at the time of calving (40.3 + 9.1 presented as percent positivity) as compared to control cows (-9.4 + 1.1). Calves that received colostrum from vaccinated cattle also had a significant increase in S. Dublin antibodies (88.5 + 8.9) as compared to calves born to unvaccinated cows (-3.2 + 1.2).

Conclusions: The results of these clinical trials indicate that the use of commercially available Salmonella vaccines can stimulate antibodies that are passed on to the calf via colostral transfer. Ongoing research is being conducted to determine whether these colostral antibodies will offer protection against Salmonella challenge. However vaccination of the dam during late gestation may provide some immunity to young calves when Salmonella challenge occurs within the first few weeks of life. The use of dry cow vaccination to provide colostral antibodies offers a way to provide some degree of protection until calves are old enough to be vaccinated.

IV-027-005

Cobalt Supplementation Affects Humoral Immune Response In Beef CalvesBob Sager ^{1,*}¹Miratorg Holdings Bryansk, Russia, Miratorg Holdings PLuskova Farm Pluskova, Russia, Russian Federation

Objectives: The objective of this study was to evaluate if a sustained release cobalt (30 gm Co O4) bolus dosed pre-weaning affects the humoral immune system post weaning and improves health during the feeding period in the feedlot.

Materials and Methods: Five different ranches with similar genetics, same forage base, mineral, same water aquifer base, and same preweaning health program were utilized in this study. Two hundred, six to eight month old steer calves (BW 220 +/- 24 kg) were randomly selected from over 2000 head. One hundred steers were randomly selected to receive a sustained CoO4 bolus at vaccination and one hundred were selected as controls. All calves were vaccinated for Mannheimia haemolytica three weeks before weaning and were bled for

existing Mannheimia haemolytica leukotoxin antibody titers at the time of treatment. All calves were weaned and transported the same day to a feedlot in Minnesota. Calves were rebled at d 70 in the feedlot. All calves in the study received the same mineral and feed rations during the feeding period and were harvested on the same day. Differences between treatment and control groups were analyzed by a Welch Two Sample t test with differences significant at $P < 0.05$.

Results: Differences between treatment and control calves showed treatment calves had an increase of 42% in Mannheimia haemolytica leukotoxin antibody ($P < 0.0586$) compared to control calves. Feedlot health (morbidity and mortality) was evaluated before weaning and during the feedlot period. Differences between treatment and control groups during the feeding period showed decreased BRD in the treatment group ($P < 0.0217$) compared with the control group. Final carcass data showed no differences in Yield nor Grade but did show an increase in HCW of 17.81 kg in the treatment group. This resulted in an increase of \$97.81 per treatment carcass after cost of treatment compared to the control group.

Conclusions: Results by this study show that supplemented Co at preweaning has potential to increase humoral immune response, decrease BRD during the feeding period, and increase profits at harvest. The sustained Co O 4 bolus supplied approximately 20 ppm of Co daily in the ration which is approximately twice NRC recommendations. Further studies need to be completed to indicate desired recommended levels.

IV-027-006

There is a need to raise farmers' awareness of correct vaccine storage temperatures

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Objectives: According to label requirements vaccines are often stored on farm in a fridge (2 and 80C) prior to being administered. Failure to maintain correct storage temperature has been shown to compromise vaccine efficacy and indirectly, animal health. Live vaccines are more sensitive to potency loss at elevated temperatures. Inactivated vaccines are typically more stable to moderate heat exposure, but more sensitive to freezing. The aim of this study was to monitor the temperature of farm fridges used to store both live and inactivated animal vaccines.

Materials and Methods: Between January and August 2014, temperature data loggers (EL-USB-2-LCD, Lascar Electronics, UK) were placed on 19 farms in south-west England. On each farm 3 data loggers were placed, 2 inside the farm fridge used to store vaccines and 1 in the room in which the fridge was situated. The loggers were set to record the temperature every 30 minutes. Data logger temperature recordings were analysed using Microsoft Excel 2010 (Microsoft Corporation). For each data logger, individual temperature recordings below 20C or above 80C were identified. The recordings for each data logger were further categorised into "Events". An Event occurred when 2 or more consecutive individual recordings were below (or above) a defined ($>80C$, $<20C$ and $=$ or $< 00C$) temperature parameter. For each Event, the minimum (or maximum) temperature was recorded, as well as the duration.

Results: Of the 19 farms, complete data for analysis were available from the fridges on 17 farms (8 dairy, 2 pig, 4 sheep and 3 beef/sheep). None of the fridges maintained the internal temperature between 2 and 80C during the study period. Sixteen fridges had at least 1 individual fridge temperature recording above 80C, the maximum fridge temperature

recorded being 240C. There was an increase in the number of fridges with a recording above 80C between May to August, suggesting an impact of higher environmental temperature. Of these 16 fridges, 14 had at least one Event, where the fridge temperature was sustained above 80C for at least 30 minutes. Eleven fridges had at least 1 individual temperature at or below 00C, the minimum fridge temperature recorded being -120C. The number of fridges with a recording at or below 00C did not alter on a monthly basis during the study. Of these 11 fridges, 7 had at least one Event where the temperature was sustained at or below 00C for at least 30 minutes, providing an opportunity any vaccine vial contents to become frozen.

Conclusions: All of the fridges in this study failed to maintain the temperature range required to store vaccines correctly. In a significant number of fridges, the temperature was outside the required storage range for a long enough period of time to produce a loss of vaccine potency. This may have a negative impact on both vaccine efficacy and animal health. There is a need to increase awareness amongst farmers of correct vaccine storage temperatures. This could be achieved with on-going monitoring to ensure this is complied with.

IV-027-007

Intranasal Vaccination in the Face of Maternal Antibody Induces Long-term Mucosal Immune Memory

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Objectives: Intranasal (IN) vaccination of newborn calves with a modified-live viral (MLV) vaccine is an effective strategy to avoid vaccine interference by maternal antibody and induce mucosal immunity with local production of IgA (Hill et al. 2012). The objective of the present study was to determine if vaccination of young calves, in the face of specific maternal antibody (IFOMA), with an IN MLV bovine herpesvirus-1 (BHV-1) and parainfluenza virus-3 (PI-3) vaccine would induce protective immune memory, compared to a MLV and a killed viral (KV) vaccine given intramuscular (IM).

Materials and Methods: Three groups ($n = 30$ /group) of Angus-cross suckling beef calves, between 3 to 6 weeks of age, were vaccinated either with a commercial MLV, BHV-1/PI3 vaccine IM, or IN, or with a KV BHV-1/PI3 vaccine IM. Control calves ($n = 20$) were injected IN with vaccine diluent. Serum samples were collected from all calves at the time of vaccination to assay BHV-1 and PI-3 maternal antibody titres. Serum samples were collected again when calves were 5 months old to assay antibody titres and 20 calves/group were selected that were seronegative for both BHV-1 and PI3. Calves were weaned at 6 months of age and the selected seronegative calves received either no vaccination ($n = 10$ /group) or a secondary vaccination matching the primary vaccination ($n = 10$ /group). Four days after vaccination all calves were aerosol challenged with 5×10^7 plaque forming units (pfu) of a clinical BHV-1 isolate (isolate 108). Calves were monitored daily for 10 days for clinical signs of disease (fever, weight loss), virus shedding in nasal secretions, interferon-gamma (IFN-g) secretion in nasal secretions, and BHV-1-specific antibody responses.

Results: The assay of serum VN antibody titres confirmed that all calves had high levels of maternal antibody when vaccinated at 3-6 weeks old but antibody was no longer detectable at 5 months of age. Following BHV-1 challenge, the naïve Control group shed detectable levels of

BHV-1 on all days post-infection (pi) and virus shedding exceeded 10⁶ pfu/ml of nasal secretion on day 5 pi. These calves also displayed a significant ($P < 0.05$) increase in body temperature between day 2 to 5 pi and a significant decrease in body weight between day 2 to 10 pi. Elevated IFN-g levels (>9000 ng/ml) were detected in nasal secretions on days 3 and 6 pi. In contrast, calves receiving an IN vaccination at 3-6 weeks and 6 months of age displayed no significant increase in body temperature, did not lose weight, and shed less than 10² pfu of BHV-1/ml of nasal secretion. Calves receiving one or two IM injections of the KV vaccine displayed similar fever, weight loss, and virus shedding as naïve controls. Calves receiving one or two IM injections of the MLV vaccine had a significant decrease in fever and weight loss but shed a similar level of virus in nasal secretions when compared to the naïve controls. Relative to the naïve controls, all vaccinated calves displayed significantly lower IFN-g levels in nasal secretions and significantly increased BHV-1-specific serum antibody responses on day 10 pi.

Conclusions: Our study shows only the IN vaccination of young calves IFOMA induced sufficient immune memory to support rapid induction of a protective secondary immune response 4 days prior to BHV-1 challenge. Changes in the IFN-g and serum antibody responses support the conclusion IM injection of KV and MLV vaccines IFOMA can induce immune memory. However, this immune memory was insufficient at 5-6 months of age to support the rapid induction of a protective immune response. In conclusion, IN but not IM vaccination, with a MLV BHV-1/PI-3 vaccine IFOMA can be combined with a secondary IN vaccination at weaning to induce protective immunity < 4 days.

IM-032-001

Evaluation of a computer-aided lung auscultation system for diagnosis of bovine respiratory disease in feedlot cattle

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Objectives: A computer-aided lung auscultation system (CALA; Whisper stethoscope, Geissler Corporation, Minneapolis, USA) was recently developed to diagnose bovine respiratory disease (BRD) in feedlot cattle.

The objectives were (i) to determine, in a case-control study, the level of agreement between CALA and veterinary lung auscultation and (ii) to evaluate the sensitivity (Se) and specificity (Sp) of CALA to diagnose BRD in feedlot cattle.

Materials and Methods: 561 Angus-cross steers (initial body-weight = 246 ± 45 kg) were observed during the first 50 days after arrival at a feedlot. Steers with visual BRD signs (identified by pen checkers) were examined by a veterinarian, including lung auscultation using a conventional stethoscope and CALA that produced a lung score from 1 (normal) to 5 (chronic). For each steer examined for BRD, 1 apparently healthy steer was selected as control and similarly examined. Agreement between CALA and veterinary auscultation was assessed by kappa statistic. CALA's Se and Sp were estimated using Bayesian latent class analysis.

Results: Of the 561 steers, 35 (6.2%) were detected with visual BRD signs and 35 were selected as pen-matched controls. All steers with visual BRD signs had abnormal lung sounds (i.e. increased bronchial sounds and/or crackles and/or wheezes) detected by veterinary auscultation. Interestingly, 9 steers selected as controls had also abnormal lung sounds. A CALA score was obtained from all examined steers (n = 70), with scores ranging from 1 to 5. Comparison of CALA results with veterinary auscultation (using a CALA score ≥ 2 as a cut-off) revealed a substantial agreement (kappa = 0.77; 95% CI, 0.62-0.92), with 62 concordant results out of the 70 clinical examinations. The 8 discordant results were attributed to the presence of abnormal lung sounds (i.e. increased bronchial sounds and/or crackles and/or wheezes) detected by veterinary auscultation, but not by CALA. Using latent class analysis, CALA had a relatively high Se (92.9%; 95% credible interval [CI] = 0.71-0.99) and Sp (89.6%; 95% CI = 0.64-0.99) for diagnosing BRD compared to pen checking.

Conclusions: CALA had good diagnostic accuracy (albeit with a relatively wide CI). Based on the relatively high specificity for CALA, we inferred that this technology has the potential to significantly decrease the proportion of cattle falsely diagnosed with BRD and thus, could promote prudent use of antimicrobials in commercial feedlots (by reducing unnecessary treatments).

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Objectives: The objective of this study was to identify simple potential prognostic indicators amongst the down cow population presented to the Large Animal Clinic at the Faculté de Médecine Vétérinaire at Université de Montréal (CHUV).

Materials and Methods: A retrospective study of recumbent cows (RC) admitted to the Large Animal Clinic at the Faculté de Médecine Vétérinaire at Université de Montréal (CHUV) was conducted. The medical files were reviewed and the following variables were extracted: number of cases per year, survival, days of recumbency prior to presentation, number of days of hospitalization, number of days of flotation therapy (FT), clinical diagnose(s), serum biochemistry profile parameters (Aspartate Aminotransferase (AST), Creatine Kinase (CK), Calcium (Ca), Potassium (K), Phosphorus (P), Carbon Dioxide (CO₂) and Betahydroxybutyrate (BHB)). Mean, median and 95% confidence intervals (CI) were calculated for continuous variables. Mean values for survival (SC) and non-survival cows (NSC) were compared with Z test. Biochemistry parameters were categorized according to their reference values into low, normal and high. Chi-2 test was used to evaluate the relationship between these categorized parameters and the survival. All differences were considered significant if P<0.05

Results: A total of 1116 RC were admitted to the CHUV between March, 1995 and June, 2014. The number of days of recumbency varied from 1 to 22 days (mean=4.5, 95%CI: 4.2-4.7). The mean number of days of recumbency at the farm was not different between SC (4.5 days; 95%CI: 4.2-4.8) and NSC (mean: 4.4 days; 94%CI: 4.1-4.8).

Among all the cases, 565 cows (50.6%) survived. The number of days of hospitalization varied from 1 to 55 days (mean=7.7 days; 95%CI: 7.3-8.1). A total of 976 cows (87.5%) received FT. Duration of FT varied from 1 to 22 days (mean=3.2 days; 95%CI: 3.1-3.4). Mean number of days of FT was not different between SC (mean: 3.3 days; 95%CI: 3.1-3.5) and NSC (mean: 3.1 days; 95%CI: 2.9-3.4).

A serum biochemistry profile was performed on 1028 cows (92.1%). The mean AST for NSC (n=465) was 855 IU/L (95%CI: 784-925) and for SC (n=523), it was 489 IU/L (95%CI: 447-531). The mean CK for NSC (n=459) was 24 175 IU/L (95%CI: 20923-27427) and for SC (n=518), it was 11705 (95%CI: 9919-13491). In general, values of BHB were significantly higher among NSC than SC, while CO₂ was significant higher among SC than NSC. Values of Ca and K were not statistically different between SC and NSC.

The relationship between the biochemistry results by category and cow's survival was not statistically significant for Ca, P, and K (P>0.05). However, a statistically significant association between categorized results and cow's survival was observed for CO₂ (Chi-2=8.8; P=0.012) and BHB (Chi-2=6.3; P=0.043). When CO₂ result was normal or high the proportion of survival cows was higher (53% and 57%).

Conclusions: Establishing a prognosis remains a challenge. Some basic clinical parameters are associated with a reduce chance of survival however each case remains unique and a methodical, rigorous, and systematic approach is the only way to increase the chances of success. Down cow syndrome remains a frustrating problem for the veterinarian practitioner. New ways to assist in lifting the cows could lead to better evaluation, more accurate diagnosis, and ultimately improved accuracy of prognosis

IM-032-002

Retrospective study of cases treated with flotation therapy at the Large Animal Clinic at the Faculté de Médecine Vétérinaire, Université de Montréal

IM-032-003

Anaplasmosis, an underdiagnosed disease in Irish Cattle Herds

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Objectives: Review of the clinical presentation of anaplasmosis from a herd which the author was involved in investigating and treating. Additionally diagnostic test results are reviewed for anaplasmosis tests at FarmLab Diagnostics.

Materials and Methods: Testing was carried out on samples which were submitted for diagnostic testing at FarmLab Diagnostics. Samples originated from a variety of Irish veterinary practices including the practice where the author is a partner. Submitting Veterinary Practitioners requested that samples be tested for Anaplasmosis using RT-PCR. Multiple samples from the same farm were pooled before DNA was extracted. DNA was extracted from EDTA blood samples by EZNA Blood DNA extraction kit (VWR, Ireland). The multiplex TaqMan PCR was performed with specific primer sets on Mx3005P Thermocycler (Agilent Technology).

Results: Laboratory investigation of a dairy herd showed that anaplasmosis was responsible for a clinical syndrome involving pyrexia and milk drop over a prolonged period. Approximately 25% of laboratory submitted samples in suspect cases, tested positive for anaplasmosis. Samples were tested using specific PCR primers to determine the species of Anaplasma involved. Approximately half, tested positive using Anaplasma marginale specific primers, while the remainder tested positive using Anaplasma phagocytophilum primers

Conclusions: The clinical importance of Anaplasmosis in Irish cattle herds is likely to be underestimated, it should be considered as a differential diagnosis of milk drop syndrome. RT-PCR testing of individual or pooled blood samples provides a highly sensitive and specific means of diagnosis. Further investigation on the significance of this disease may be warranted.

IM-032-004

Inter-rater agreement and diagnostic accuracy of lung auscultation to detect calf pneumonia

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Objectives: A more rational use of antimicrobials for bovine respiratory disease (BRD) would imply only antimicrobial treatment of animals with a bacterial pneumonia and not systematically in upper respiratory tract infections. Lung auscultation is traditionally used to diagnose pneumonia in a calf with respiratory signs. Information on agreement of lung auscultation between different veterinarians is not available. Also its diagnostic accuracy compared to thoracic ultrasonography, the most sensitive and specific technique to detect pneumonia available in practice, is not determined.

Materials and Methods: The study was conducted in three Dutch veal farms in three different sessions on consecutive days. A calf pen which was recently diagnosed by the farmer as suffering from BRD was selected beforehand. Participants were asked to auscultate the

lungs of all calves present in this pen (8-10), as they daily perform in practice. They reported their findings as a binary variable (normal vs. pneumonic) to a recorder located outside the pens. Veterinarians were only allowed to auscultate and specifically asked not to perform other clinical examinations. Afterwards all calves were examined by thoracic ultrasonography with a 7.5 MHz linear probe (Mylab One, Esaote) by a single operator. Pneumonia was defined as a consolidation of >1 cm². Kappa statistics, Cohen's and Fleiss's, were calculated to estimate agreement of auscultation with ultrasonography and inter-rater agreement, respectively. Diagnostic accuracy of lung auscultation was determined by calculation of sensitivity (Se) and specificity (Sp) with ultrasound as gold standard.

Results: A total of 49 Dutch vets participated in three different sessions. The % of calves with consolidations was 63.0%, 78.0% and 60.0% in sessions 1 to 3, respectively. The mean kappa value for the agreement of auscultation with ultrasonography was 0.38 ± standard deviation (SD)= 0.22 (Range (R)= 0.10-1.0), which is only 'fair' according to Cohen's classification. This agreement was not influenced by session, gender or years after graduation of the participants. Average Se and Sp of lung auscultation were 0.63 (SD= 0.2; R= 0.2-1.0) and 0.46 (SD= 0.3; R= 0.0-1.0), respectively. Of the participants, 8.2% was 100% sensitive, 16.3% was 100% specific, and only 4.0% was perfect. Inter-rater agreement, expressed by Fleiss Kappa, was 0.17 (95% confidence interval (CI)= 0.13-0.22), 0.24 (95% CI= 0.19-0.29) and 0.06 (95% CI (-0.03-0.16)) for sessions 1 to 3, respectively. These values signify a poor inter-rater agreement.

Conclusions: In conclusion, the average low Se and Sp of lung auscultation and only fair agreement with ultrasonography do not support its use as a definite criterion to confirm pneumonia and initiate antimicrobial treatment in a veal setting. Despite that some veterinarians were highly accurate, the variation between veterinarians was extremely large. Better training in lung auscultation or alternatively the use of more standardized techniques such as ultrasonography or electronic stethoscopes is recommended.

IM-032-005

Acute Bacterial Pleuropneumonia in adult dairy cows: A retrospective study of post mortem cases

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Objectives: Feedback from practitioners suggests that respiratory disease in adult dairy cows is becoming a more common clinical presentation in practice. In some situations the clinical signs can be severe and acute, with a poor treatment response.

The disease syndrome has a varied potential aetiology including infectious agents and a range of potential animal, management and environmental risk factors.

The aim of this study was to review cases of acute bacterial pleuropneumonia (ABP) examined at post mortem to gain a better understanding of the aetiology and management of this condition.

Materials and Methods: 26 cases of ABP in adult dairy cows based on a gross pathological case definition were examined post mortem at Scottish Disease Surveillance Centres between 2005 and 2015. A standard case definition was used and cases where other diagnoses of respiratory disease were made were excluded from the study.

At post mortem a standard tissue set was collected from each case to allow bacterial and mycoplasma cultures, respiratory virus detection by PCR and histopathology.

Herd and clinical case histories were collected using a standard pro-forma and clinical information was provided by the referring veterinary surgeon.

This descriptive data was reviewed and is summarised in this presentation.

Results: Cases were received from larger, open, expanding dairy herds with some or all cattle housed. It is acknowledged however that these farms are typical of those seen in the area.

There was no seasonal or age trend in affected cattle and 50% of cases occurred within 2 months of calving. All cows were treated with antibiotics prior to either dying or being euthanased on welfare grounds.

The clinical signs were acute, with severe or complete milk drop, anorexia, pyrexia, tachypnoea and severe dyspnoea.

The pathology seen in all cases was an acute fibrinous, bronchopneumonia with pleurisy. Histopathology confirmed an acute suppurative pneumonia consistent with a bacterial aetiology. *Mannheimia haemolytica* and *Pasteurella multocida* were the most common bacterial isolates. No evidence of viral involvement was detected in these cases either by virus detection methods or histopathology.

Potential risk factors were identified on each farm, although these were not tested by a case control study approach. Risk factors included poor housing ventilation, overstocking, poor ventilation of collecting yards, biosecurity, poor transition cow management, heat stress and a previous episode of calf pneumonia.

Conclusions: It was concluded that ABP is a significant disease problem in affected herds and a welfare problem for affected cattle. It is of note that the pathology seen was consistent with a primary bacterial aetiology and no evidence of viral involvement was detected. This information along with potential risk factors identified could allow the practitioner to focus on critical areas for control and prevention of this disease syndrome.

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LA-035-001

The Effect Of Lameness Before And During The Breeding Season On Fertility In 10 Pasture-Based Irish Dairy Herds

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Objectives: The effects of lameness on fertility have been documented frequently but few data are available from seasonally breeding, pasture-based herds (such as those used in Ireland) where cows are housed during the winter months but managed at pasture for the remainder of the year. This study determined the prevalence of lameness in a group of 786 cows in 10 pasture-based Irish dairy herds before, during and after the breeding season and assessed the relationship between lameness and the reproductive performance in these herds through serial locomotion scoring during the grazing period.

Materials and Methods: This prospective observational study was carried out in 2013 on 10 commercial Irish dairy farms, as part of the on-going herd health management programme conducted by University College Dublin (UCD). All the farms used seasonal breeding and were visited by a UCD veterinarian every 21 days during the calving and breeding season. Reproductive performance was monitored at a herd-level based on Submission rate, Conception risk and Pregnancy rate. Lameness data were gathered by means of serial locomotion scoring of all the animals in the spring breeding herd. Four locomotion scoring visits were scheduled in accordance with the breeding season to allow for cows to be locomotion scored before, during and after the breeding season. Kaplan-Meier survival curves were used to compare the PR distribution and to calculate median days to conception. A Cox proportional hazards model with lameness as time varying covariate was used to identify variables influencing the PR.

Results: Lameness prevalences of 11.6% before, 14.6% during and 11.6% after the breeding season were found. The median days to conception was 26 for cows never observed lame, 33 for cows lame before the start of breeding but no longer lame during the breeding season, 36 for cows that became lame during the breeding season and 43 for cows lame both before and during the breeding season. After controlling for the effect of, farm, month of calving, body condition score at calving, body condition score loss after calving and economic breeding index in the Cox proportional hazards model, cows identified as lame during the study were less likely to become pregnant. Cows lame before the earliest serve date but no longer lame during the breeding season, cows becoming lame after the earliest serve date and cows identified lame both before and after this date were respectively 12%, 35% and 38% less likely to become pregnant compared to cows never observed lame during the study. However, these findings were only significant for cows becoming lame after the earliest serve date and cows lame both before and after the start of breeding.

Conclusions: The lameness prevalence found in this study compared favourably to results from housed cattle and is similar to other studies carried out in grazing herds. The reproductive efficiency was significantly ($p < 0.05$) lower in cows becoming lame during the breeding season and cows lame before and during the breeding season compared to non-lame cows. The PR was not significantly ($p > 0.05$) lower in cows no longer lame during the breeding season compared to cows never diagnosed as lame. In addition to lameness status, nutritional status and genetics were found to influence the reproductive performance in pasture-based Irish dairy herds.

LA-035-002

Applying A Scientific Based, Standardized Footbath Protocol: The Impact On Digital Dermatitis Dynamics

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Objectives: Digital dermatitis (DD) is the most prevalent foot lesion in Canada. The most common on-farm prevention strategy is the use of footbaths. However, there is a wide variability in on-farm practices related to footbath management; including dimensions, frequency of use, products used and concentration. Despite a wealth of information regarding effective practices in the scientific literature, there seems to be a knowledge translation gap between research and on-farm application. Therefore, the objective of this study was to evaluate the effectiveness of a science-based standardized footbath protocol in the field as a preventive measure for DD.

Materials and Methods: A longitudinal field trial was conducted on 9 Alberta dairy farms over 7 months. Each farm served as its own historical control, where 1,300 cows were evaluated before and after the introduction of a new footbath and protocol. The intervention consisted of implementing a computerized automated footbath on all farms using a standardized protocol based on literature recommendations of footbath design (300 cm long x 50 cm wide x 15 cm high), product (CuSO₄), concentration (5%), and frequency (4 milkings). A computerized program dictated the automated mixing of CuSO₄ at a 5% concentration during 4 consecutive milkings on a weekly basis and refreshed the footbath solution every 200 cow passes or less.

DD scoring was done in the milking parlour (every 2 weeks) and confirmed with the gold standard of hoof trimming observations (at the beginning, half-way and end of the study) using the M-stage scoring system and interpret as lesion, active lesion and chronic lesion. In cases with more than one lesion per foot or cow, the most severe lesion was considered for analysis according to the proposed hierarchy of M2 > M4.1 > M4. Initial results were analyzed using paired T-tests. Survival analysis will be applied for a better insight in the preventive effect of the standardized footbath protocol.

Results: Controlling footbath dimensions, along with CuSO₄ concentration and frequency of use, and refreshment of solution, resulted in a change in DD dynamics. A significant decrease in the prevalence of active M2 lesions (from 21 before to 8% after intervention; $P < 0.05$) and an increase of chronic M3-M4 lesions (from 41 before to 55% after intervention; $P < 0.05$) was detected when cows were scored in the milking parlour. A similar trend was identified when cows were evaluated in the trim chute. All farms had a significant increase in the prevalence of absence of lesions (M0), from 31 before to 40% after intervention ($P < 0.05$). The prevalence of cows with active lesions decreased from 41 to 25%, whereas the prevalence of cows with chronic lesions increased from 28 to 35% (before compared to after intervention, $P < 0.05$).

Conclusions: The use of a standardized footbath protocol significantly decreased the presence of active DD lesions and increased the number of cows without lesions as observed in the trim chute. A more precise application of footbath management guidelines as recommended by scientific literature can result in control of DD dynamics to a steady state, with a significant reduction of new cases and well-regulated presence of chronic lesions. In conclusion, controlled and standardized footbath management can result in significant reduction of the prevalence of DD.

LA-035-003

Treating claw horn lesions: Three randomised controlled trials

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Objectives: Lameness is a common condition affecting the welfare and production of cattle globally. Despite this there are few published clinical trials investigating cow level treatments, particularly of the diseases of claw horn disruption (principally sole ulcer, sole haemorrhage and white line disease). The authors have recently conducted three randomised controlled trials, two comparing treatments for lame cows with claw horn lesions and one evaluating the preventive effect of foot trimming in primiparous heifers.

Materials and Methods: The first trial was a randomised, positively controlled clinical trial comparing four treatments for newly lame cows with claw horn disease in 183 dairy cows from five farms in the UK. Cows were locomotion scored fortnightly and were eligible for recruitment if they presented with a new case of lameness in a single hindlimb. Enrolled cows were randomly allocated to receive 1. A therapeutic foot trim only, 2. A trim and foot block, 3. A trim and non-steroidal anti-inflammatory drug or 4. A trim, foot block and NSAID.

The second trial was also a randomised, positively controlled clinical trial comparing three treatments for claw horn disease in 156 cows from seven UK dairy farms. Cows were randomly allocated to receive a trim only, a trim and foot block or a trim block and NSAID. In contrast to the first trial cows were only eligible for enrolment following multiple presentations as lame at fortnightly locomotion scoring, resulting in selection of more chronically lame cows which had been locomotion score lame for at least two weeks.

The third trial was a negatively controlled randomised clinical trial evaluating the impact of an early lactation foot trim on production in 282 primiparous heifers from eight dairy farms in the UK. Heifers between 50 and 80 days in milk were randomly allocated to either untreated control groups or to a treatment group. Heifers in the treatment groups received a functional foot trim and if required a therapeutic foot trim.

Results: In the first trial, based on having a sound locomotion score 35 days after treatment the cure rates were 24.4%, 35.9%, 28.6% and 56.1% for the trim-only, trim and block, trim and NSAID, and trim block and NSAID group respectively. Compared with group 1, cows in group 4 were significantly more likely to be sound at outcome (Thomas et al, *Journal of Dairy Science*, 98(7) p.4477).

In the second trial no differences were detected between treatments and the percentage of cows locomotion scored as non-lame after 6 weeks was around 15% for all groups. This is markedly lower than the newly lame cows in the first trial. Importantly a large number of animals had become lame on the opposite hind leg i.e. the treated leg had improved but they remained lame (Thomas et al, *Veterinary Record*, In Press).

In the third trial no significant difference in 305 day milk production or 100 day in calf rates was detected between groups. Multivariable modelling identified a large increase in milk yield for heifers identified as lame at the time of enrolment in the treatment group compared to non-lame heifers in the control group. On examination, 95% of animals in the treatment group had some pathology identified on at least one claw (Maxwell et al, *Veterinary Record*, 177(4) p. 100).

Conclusions: The authors conclude that screening for, and treatment of, newly lame cows should be carried out fortnightly and cows treated for claw horn disease should receive treatment with a foot block and NSAID wherever possible. The difference in cure rate between the first

and second trial is striking, the key difference being that cows in the second trial were not treated immediately they became lame. The third study also highlights the large number of heifers with pathology at an early stage, and clearly shows a production benefit to treating those identified as lame early in lactation.

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LA-035-004

Developing A Lameness Detection Model Using Automatically Recorded Performance And Behavior Data

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Objectives: Lameness impairs welfare and performance in cows (Green, 2009, Von Keyserlingk, 2009). The increasing size of modern dairy herds leads to lower surveillance rates per individual. Additionally producers often do not recognize lame cows until they suffer from severe stages of lameness. These problems can be the reason for delayed diagnosis and as a consequence thereof substantial deprivation of the well-being of the affected animals.

In the study automatically recorded behavior and performance data were analyzed in order to detect lameness in dairy cows.

Materials and Methods: 65 Simmental cattle housed in a free stall barn were observed from April 2014 to April 2015. In order to measure individual performance (milk yield, live weight, feed intake) and behavior (feeding behavior, standing, lying and activity index) various sensors were installed. A locomotion scoring according to Sprecher et al. 1997 (1-5, 1 = healthy, 5 = severely lame) was made weekly for the entire herd to determine lame and non-lame animals. Receiver Operating Characteristic (ROC) analysis was used to find out about the parameters with the highest accuracy to predict lameness. These will be fitted in a lameness detection model.

Results: Performance parameters such as milk yield, live weight or feed intake did not show a significant change in early stages of lameness. Behavior associated parameters like feeding duration, feeding rate, duration of lying bout or total lying time were more sensitive in terms of lameness prediction. Hence these were included in the lameness detection model. Also days in milk and number of lactation were included as influencing factors.

The preliminary model applied to the recorded data classified 113 of 114 non-lame individuals and 8 of 11 lame cows correctly.

Conclusions: Lameness detection through analysis of feeding and lying behaviour can be a powerful tool to detect lameness automatically in modern free stall barns. Further research is needed to validate the findings of this project in a more significant number of individuals, different breeds and with a more practical, application-oriented environment.

LA-035-005

To bandage or not bandage: The curative effect of bandaging digital dermatitis lesions

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Objectives: Digital dermatitis (DD) is an infectious claw disease that causes lameness among cows worldwide. Efforts to eradicate DD have been overly focused on treatment and less so on standardized maintenance practices, like bandaging, to enhance the effects of treatment. Very few studies have compared the effectiveness of bandaging to non-bandaging. In an attempt to examine the fit of bandaging in the treatment of DD, this study will examine the effect of bandaging on wound size and locomotion among a sample of dairy cows receiving either antibiotic or non-antibiotic treatment.

Materials and Methods: Animals

This randomized clinical trial study included (N=162) Holstein Friesian dairy cows, diagnosed with ulcerative DD lesions (M2) upon the first examination (week 0). Cows, ranging from heifers to cows in their 4th lactation, were housed in a stable fitted with cubicles and with concrete flooring.

Treatment and Evaluation

All hoofs were cleaned and trimmed by a professional hoof trimmer or a veterinarian. The M2 lesions of cows in the first arm of the study (N=85) were sprayed with CTC, a topical treatment containing chlortetracycline (WdT, Garbsen, Germany). Cows were then randomly assigned into either a non-bandaged (N=41, 48%, Group 1) or bandaged group (N=44, 52%, Group 2). A topical non-antibiotic gel, containing activated copper and zinc chelate (Intra Hoof-fit gel [IHF], intra Care b.v), was applied to the M2 lesions of cows in the second arm of the study (N=78). Cows were then randomly assigned into either a non-bandaged (N=40, 51%, Group 3) or bandaged group (N=38, 49%, Group 4). The bandaging process was standardized and applied by the same veterinarian for all groups.

The process of wound healing was evaluated and scored once weekly (weeks 0, 1, 2, 3, 4) according to a visual inspection scheme described by Döpfer et al., 1997 and Berry et al., 2012. Photographs of lesions were taken and later, under the use of a special software package (Jalomed®), were used to track lesion size across observations. The healthy formed skin (M0) was judged as full recovery. Locomotion was also evaluated and scored once weekly according to Sprecher et al., (1997) locomotion scheme.

Results: For cows treated with CTC, (82%, n=38, Group 2) of bandaged and (44%, n=18, Group 1) of non-bandaged cows were deemed macroscopically healed at week 4. A Survival Analysis concluded that healing was significantly higher for bandaged than non-bandaged cows following topical antibiotic treatment ($Z = 4.653$, $p < 0.001$, 95% CI: 2.19 to 6.84). Furthermore, bandaged lesions were significantly less likely to transition into M4 lesions, the chronic digital dermatitis ($p < 0.001$). For cows treated with IHF, (73%, n=27, Group 4) of bandaged and (30%, n=12, Group 3) of non-bandaged cows were deemed macroscopically healed at week 4. A Survival Analysis indicated that healing was significantly higher for bandaged than non-bandaged cows following topical non-antibiotic treatment ($Z=3.352$, $p < 0.001$, 95% CI: 1.627 to 6.403). Moreover, bandaged lesions were significantly less likely to transition into M4 lesions, the chronic digital dermatitis ($p < 0.001$). A Wilcoxon Rank Sums Test indicated that bandaging had no effect on locomotion for either cows treated with CTC ($W = 13601$, $p < 0.27$, CI:

-4.58e-05 to 1.77e-05) or IHF ($W = 14369$, $p < 0.332$, CI: -4.02e-05 to 5.77e-05). However, wound size was significantly larger for cows with locomotion scores between 3 and 5 than for cows with lower scores for both CTC ($W = 8621$, $p < 0.019$, 95% CI: -2.08 to -0.03) and IHF treatment groups ($W = 8051$, $p < 0.001$, 95% CI: -2.53 to -0.68).

Conclusions: Results suggest that bandaging accelerated the healing of DD lesions, regardless of treatment type. Bandaged lesions were significantly less likely to develop into stage M4, the chronic stage of digital dermatitis. Thus, our study indicates that covering DD lesions is advantageous to both the wound healing process and the cow's wellbeing.

LA-035-006

A randomised clinical trial on the effect of antibiotic or non-antibiotic topical treatment of digital dermatitis in dairy cattle

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Objectives: Digital dermatitis (DD) is a superficial, painful, contagious epidermitis of the feet of dairy cattle which frequently results in lameness. A common individual treatment for DD in Europe is a topical administration of antibiotic chlortetracycline (CTC) spray. Given potential induction of antibiotic resistance there is a need for effective treatments without antibiotics. We performed a randomised controlled trial to compare clinical improvement of ulcerative DD lesions following treatment with IR spray versus CTC spray (non-inferiority testing).

Materials and Methods: We included 9 dairy herds of around 100 cows housed in a freestall system with an estimated DD prevalence of 20-25%. In total, hind legs of 944 cows from 9 dairy herds were trimmed by professional hoof trimmers and scored using the M1-M4 scoring system. All legs with M2 lesions were included and randomly treated with IR spray or CTC spray according to the instructions of the manufacturers. Clinical improvement was defined as the transition of an ulcerative M2 lesion to any other lesion at treatment evaluation on day 10. At the end of the study 231 cows, of which we included one M2 affected leg each, were eligible for analysis.

Results: After treatment with IR spray, most lesions (71%) transitioned into an M3, while the percentage of M1 and M2 lesions was almost equal (respectively 14% and 13%). After treatment with CTC spray most lesions remained M2 (52%). The percentage of M1 lesions after treatment with CTC spray was a little higher compared to treatment with IR spray (19%). Only 1% and 3% of M2 lesions for respectively IR spray and CTC spray transitioned to M0. The overall clinical improvement rate of IR spray was higher compared to CTC, respectively 86.8% (range 61.5% - 100.0%) and 47.9% (range 17.7 - 85.2%). In all 7 herds clinical improvement was numerically higher for IR spray and in 3 out of 7 herds significantly higher. The Odds Ratio, adjusted for herd effects, for IR spray versus CTC spray was 8.2 (95% CI 4.2 - 15.7) with an estimated Relative Risk of 1.9.

Conclusions: Overall the non-antibiotic IR protocol results in a greater clinical improvement rate than the antibiotic CTC protocol with an Odds Ratio of 8.2 (95% CI 4.2 - 15.7) and an estimated Relative Risk of 1.9. Clinical improvement rates varied between herds, but was numerically higher on all farms for IR spray. The large variation between herds suggests that differences in environmental conditions affect clinical cure.

LA-035-007

Gait cycle in dairy cows and its application to detect lamenessMaher Alsaad^{1,*}, Thomas Hausegger², Ralf Kredel², Adrian Steiner¹¹Clinic for Ruminants, Vetsuisse-Faculty, University of Bern, ²Institute of Sport Science, Faculty of Human Sciences, University of Bern, Bern, Switzerland

Objectives: This study evaluated the feasibility of high frequency accelerometers (400 Hz) to measure gait cycle parameters of stance and swing phases in dairy cows. It was hypothesized that an accelerometer with a high sampling rate (400 Hz) allows for (i) accurate identification and description of the gait cycle pattern in cattle, including the duration and amplitude of swing and stance phases, compared to a high speed camera output, and (ii) a clear distinction between healthy and affected limbs in lame cows.

Materials and Methods: The experimental study was carried out at the Clinic for Ruminants, Vetsuisse-Faculty, University of Bern, Switzerland. Twelve dairy cows without any visible signs of lameness (C) and five lame cows (L) referred to the clinic were used. The measurements of acceleration for groups C and L were conducted during walking in two sessions: Session (I) assessed the accelerations of the metatarsus (MT) and lateral claw of the same limb simultaneously; session (II) assessed the acceleration of the MT of both hind limbs simultaneously to compare the accelerations between left and right MT and calculate the difference for each variable separately (Δ). The extracted gait cycle parameters included (i) several amplitudes (preswing, swing, foot load, ratio of preswing to foot load and the ratio of swing to foot load phase) and (ii) temporal events (relative duration of stance phase and swing phase to gait cycle and of preswing to stance phase).

Results: In session (I), the amplitude of preswing, swing, foot load, ratio of preswing to foot load and ratio of swing to foot load of the lateral claw was significantly higher as compared to the respective MT ($P < 0.05$). The temporal events of the gait cycle, including the relative duration of stance and swing phases of the claw were highly correlated with the MT ($r_s > 0.71$), and only the relative duration of the preswing to stance phase was significantly higher at the level of the MT as compared to the claw ($P < 0.05$).

In session (II), amplitudes and relative temporal events of the gait cycle were not significantly different and moderately to highly correlated ($r_s : 0.52-0.95$) between left and right MT in group C. Δ of the amplitude of foot load, ratio of preswing to foot load and ratio of swing to foot load were significantly higher in group C as compared to group L ($P < 0.05$). All the Δ of the temporal events of the gait cycle were significantly different between group C and group L ($P < 0.05$).

Conclusions: Measuring the gait cycle variables at the level of the MT, using accelerometers with a high sampling rate (400 Hz) is a promising tool to indirectly explore the acceleration of the claw and determine the effect of orthopedic pathologies on the cows' gait.

MC-013-001

A Screening Method To Significantly Reduce The Use Of Intramammary Antibiotics At Drying Off In Dairy Cow Production

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Objectives: Antibiotics used in agriculture contribute to the development of antimicrobial resistance (Landers et al., 2012) and are the subject of controversy in public opinion. A selective dry cow therapy programme would enable a significant reduction in the use of antibiotics in dairy production. Milk Amyloid A (MAA) has been suggested in several studies as a biomarker of both clinical and subclinical bovine mastitis (Gerardi et al., 2009; Pyörälä et al., 2011). We conducted a field study to evaluate the efficiency of the measurement of MAA when used to make selective antimicrobial treatment decisions at mammary quarter level on cows at drying off.

Materials and Methods: Individual mammary quarter milk samples from 112 cows, originating from low bulk tank somatic cell count (SCC) (<250,000 cells/mL) dairy herds (n=6), were collected between two to seven days prior to drying off (n=445) and after calving (n=339), starting from two weeks until six weeks. All milk samples were cultured for bacterial detection and identification. They were analyzed for SCC and for MAA concentration using a patented commercial ELISA (Tridelta Development Ltd, Maynooth, Ireland). We performed a selective dry cow therapy at quarter level based on MAA results. The quarters from cows with an MAA concentration < 1 µg/mL were not treated with an antibiotic therapy. They were only infused with a teat sealant (n=92). We developed an algorithm to identify an intramammary infection (IMI) at mammary quarter level at the end of lactation based on the gathered MAA and SCC results. The test characteristics of the algorithm were calculated and its negative (NPV) and positive (PPV) predictive values were estimated using the formulas based on Bayes' theorem. Cow/milk data and bacterial culture/detection data were used as a gold standard. Our algorithm was compared with the SCC recording prior to drying off. A threshold of 100,000 cells/mL for primiparous cows or of 150,000 cells/mL for multiparous cows to identify an IMI was used for the SCC test.

Results: The sensitivity and specificity of the developed algorithm were both high, respectively 94.5% and 93.0% and the PPV and NPV in our study population were respectively 96.3% and 89.9%. By contrast, the sensitivity and the NPV of the SCC test were low, respectively 68.7% and 62.8%. As a result, our algorithm could be used to make selective dry cow treatment decisions at quarter level with the confidence that very few infected mammary quarters would be missed and that high proportion of the healthy mammary quarters would be not treated with antibiotic therapy. In contrast, the SCC recording just prior to drying off seems unsuitable as part of this selective programme.

The algorithm's predictive values were both above 90% when estimated in herd populations where the proportion of mammary quarters with an IMI at drying off ranged from 40% to 65%. Similar but more moderated results were obtained for a prevalence ranging from 30% to 75%. Consequently, in these various herds, the proportion of quarters truly infected with a negative test that would not receive an intramammary antibiotic treatment would be low (1 - NPV < 10%) and few quarters truly healthy with a positive test sentence (1 - PPV < 10%) would be treated with an antibiotic therapy.

Moreover, we didn't observe any clinical mastitis six weeks after calving for quarters with a negative algorithmic test that were not treated with antibiotics and were only infused with a teat sealant. These quarters have achieved success in the treatment and prevention of IMI over the dry period.

Conclusions: The use of our screening method for an IMI, based on an algorithm which utilizes MAA and SCC results measured in individual quarter milk samples, in a selective dry cow therapy programme at the quarter level would allow a significant reduction in the use of intramammary antibiotics in dairy production with confidence. In our study, its application would have reduced the use of antibiotic treatments by 29%.

In France since September 2015, we offer a service at drying off for dairy farmers based on our method. The gathered data (MAA, SCC, milk/cow data, bacterial data) are used to grow our database that is used to support our algorithm.

MC-013-002

The attitude of Dutch veterinarians towards antimicrobial use in dairy cows

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Objectives: Prudent use of antimicrobials in animal husbandry is of major importance in order to reduce the risk of development of antimicrobial resistance. Although on a dairy farm farmers apply most of the antibiotics, the veterinarian as the main advisor on this subject has an important role and responsibility. Understanding the attitude of veterinary practitioners towards antimicrobial use and selective dry cow therapy (SDCT), is crucial in a successful approach of responsible antimicrobial use in dairy practice. The aim of the present study is to describe the attitude of Dutch veterinarians towards antimicrobial use and SDCT on dairy farms.

Materials and Methods: The mindset of veterinarians towards antimicrobial usage in dairy practice and the implementation of SDCT was evaluated retrospectively using a detailed questionnaire. A group of 648 Dutch cattle veterinarians received the questionnaire by e-mail using the software program Survey Monkey. The questionnaire contained items about mindset regarding antimicrobial use, positive and negative aspects of the reduction of antimicrobial use, criteria for selecting cows for SDCT and demographic data. There were open questions, questions with pre-defined answer categories, as well as statements that the farmers rated on a 5-point Likert scale. Results of the questionnaires were summarized and analyzed descriptively to explore the data.

Results: Of the questionnaires sent, 207 (32%) were returned and suitable for analysis. With regard to advice about prudent and restricted use of antimicrobials in general, 71% of the veterinarians see themselves as the main technical advisor, and 56% of the veterinarians are of the opinion that they play a proactive role in the changed policy of restricted use of antimicrobials. A minority of the veterinarians accredit their role negatively; 15% of the veterinarians only see themselves as the messenger of bad news, whereas 9% considers themselves as solvers of a problem the farmer has to handle.

Most of the veterinarians (70%) speak positive about SDCT. The majority of the veterinarians (88%) indicated they judged themselves as being on the right page, with regard to the approach of antimicrobials, and they judge 69% of their farmers is. Almost all veterinarians introduced SDCT to their farmers. Although most of the veterinarians indicated to be concerned about the negative consequences of SDCT, only a minority of them evaluated the implementation of SDCT within one year after introduction.

Conclusions: In general, the mindset of Dutch dairy veterinarians towards the changed approach of antimicrobial usage and the implementation of SDCT was positive. They feel a large responsibility regarding the implementation of the policy of antimicrobial usage and SDCT in the Netherlands.

The practitioners overestimated the problems farmers would have with introducing SDCT on their farms. They can improve their advisory skills by asking the opinion of the dairy farmers, rather than acting based on what their own thoughts are.

MC-013-003

Preliminary Results of an Investigation of the Impact of Bedding Type on Pathogen Load in Bedding, Udder Health and Milk Quality

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Objectives: There is a general lack of understanding of the impact of different bedding materials, and their management, on milk quality and udder health (Bradley et al., 2014). The aim of this study was to make a quantitative, four-way comparison of the impact of bedding type on pathogen load in bedding, milk quality, udder health and cow comfort using recycled manure solids (RMS) on mats, sawdust on mats, RMS as a deep bed and sand as a deep bed.

Materials and Methods: The study was conducted at Sewborwens Farm, Newton Rigg, Penrith, Cumbria, CA11 0AG, UK. A modified crossover design was employed using four groups of 40 cubicles within a single shed. Each group of cubicles had a different bed type: deep sand (replenished every 2 weeks), deep RMS (replenished twice weekly), RMS on mats (replenished twice weekly) and sawdust on mats (replenished twice daily). Four cow groups, each of 40 cows, were rotated around the four bedding areas, spending two weeks on each bedding type, and cycling twice around the four treatments.

Samples of unused and used bedding were collected every two weeks and a proportional bulk milk sample every week from each of the groups and submitted for microbiological analysis. Individual quarter somatic cell counts were conducted on all cows at the start and end of the study and at each change between bedding materials, allowing intramammary infection (IMI) to be defined by movements around a 100,000 cells/ml threshold. All clinical mastitis cases were sampled and cultured.

Results: There was significant variation in the bacterial load of unused bedding, with counts being significantly and consistently higher in the recycled manure solids than the other bedding materials. In used bedding, counts were typically higher in the deep and shallow RMS beds, and most frequently highest in the shallow RMS beds. The variation in bacterial numbers evident in bedding materials was not evident in milk. With the exception of *Streptococcus* spp and *Staphylococcus* spp counts, no significant differences were detected in the quality of the milk produced by animals bedded on the different bedding materials. Used sand was significantly more likely to contain *Listeria* spp than either sawdust or deep RMS and was significantly more likely to be isolated from milk from cows bedded on sand than on RMS.

The overall proportion of eligible quarters, across all two week blocks of bedding treatments, experiencing an apparent new intramammary infection or an apparent cure were calculated. There was significant variation between the bedding types ($p=0.015$). New IMIs were significantly less likely to occur in cows on sawdust beds than on deep RMS (47/961 vs 84/965; $p=0.012$) or sand beds (47/961 vs 78/965; $p=0.04$). No impact of bedding material on the likelihood of a quarter curing could be identified.

A total of 10 cows developed clinical mastitis during the 16 weeks of the study. *Klebsiella* spp were the most frequently isolated causal organism. There was a trend for cows bedded on RMS to be at higher risk of developing clinical mastitis than cows not bedded on RMS ($p=0.086$).

Conclusions: There were significant differences in the bacterial challenge to teats in different types of bedding and between bed designs when considering RMS. However, with the exception of *Streptococcus* spp and *Listeria* spp there was no clear relationship between bacterial numbers in bedding and in bulk milk, although this may, in part, reflect the hygiene practices during milking and the challenges incumbent in preparing teats on cows arriving from beds constructed with three different bedding materials. In this study, sawdust, applied to mats twice daily, appeared to offer the best protection against new intramammary infection (as measured by SCC).

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MC-013-004

Effects of antibiotic or non-antibiotic dry off treatment on milk yield, somatic cell count and frequency of clinical and subclinical mastitis

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Objectives: The use of antibiotics in dry cows is widely applied. However, concerns are becoming more evident that antibiotic dry off treatment may result in increasing resistance to antibiotics. The objective of the study was to evaluate the effect of application of long acting antibiotic treatment in comparison to cows which were dried off without antibiotic treatment using large dataset. Primary outcome parameters were milk yield, somatic cell counts and the frequency of clinical and subclinical mastitis. Further thresholds should be calculated for different parameters which should help to decide if antibiotic dry off treatment is indicated or not.

Materials and Methods: As a nationwide health monitoring system for cattle has been implemented in Austria it was possible to use the data on a large number for a retrospective analysis. A large database

was used comprising data on milk yield and health data from the dairy cattle in Austria. The diagnoses of interest for this study were "acute clinical mastitis" and "subclinical chronic mastitis". Further the records were investigated if a long-acting antibiotic dry off treatment had been administered to the cow or not. Further information like animal identification, calving date, milk yield, somatic cell count and information about the herd and the veterinarian were available. The study was conducted on Simmental cows (Fleckvieh) the predominant breed in Austria. The data was filtered and checked for plausibility. Descriptive statistics were calculated (frequency of mastitis, milk yield, somatic cell count) for the previous and subsequent lactations. A model was developed to assess the factors influencing the outcome variables frequency of clinical and subclinical mastitis, A P value < 0.05 was considered indicative for significant differences.

Results: Data of 24,135 dry off treatments and subsequent lactations from 14,301 cows were included. The data was derived from 1,170 dairy farms which contributed between 21 and 129 datasets. Among the 14,301 cows 8,193 (57.3%) were dried off without antibiotic dry off treatment and 6,108 cows (42.7%) were dried off using an antibiotic dry off treatment. The average daily milk yield during 90 DIM was 29.7 ± 6.3 kg/d for 15,516 lactations before Non-AB dry off treatment which was significantly lower than the milk yield of cows in 8,537 lactation which were 31.1 ± 6.7 kg/d which were dried of using an antibiotic treatment. In both treatment groups the average daily milk yield increased from the lactation before dry off treatment to that after. The increments between the previous and the subsequent lactation did not differ between the treatments. The somatic cell count did not differ between the groups before dry of treatment. Equally there were no differences in the subsequent lactation between the different treatments. The incidence of mastitis during lactation and during 90 DIM increased with lactation number for both treatments. The mastitis incidence was higher in the AB group during the lactation before treatment in comparison to the incidence of the non-AB group. However, this difference was still present in the subsequent lactation independently from the dry off treatments.

Conclusions: The results did not support the current belief that milk yield, somatic cell count and the frequency of clinical and subclinical mastitis during subsequent lactation differ significantly between cows which had been dried off using antibiotic treatment and cows which were dried off without antibiotic treatment. The results support the assumption that a blanket antibiotic treatment is no longer justifiable. The present study was not able to suggest cut off values We suggest that other parameters like bacterial culture or on farm culture systems should be investigated. Possibly cut offs have to be determined for each herd individually.

MC-013-005

Systematic review and meta-analysis of the effectiveness of cefquinome treatment protocols of clinical mastitis in dairy cows

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Objectives: To assess the effectiveness of two treatment protocols using cefquinome, a fourth generation cephalosporin, for the treatment of clinical mastitis in dairy cows a systematic review and meta-analysis (MA) of scientific literature was conducted. Our results will allow

evaluating the recommendation on the prudent use of cefquinome in the dairy industry.

Materials and Methods: Randomized clinical trials (RCT) were retrieved using Medline, Science Direct, Web of Science, Cab Abstracts, and Scopus databases. In addition, conference proceedings, the list of references of the included studies, and direct contact with authors were also used as a study search strategy. Studies considering lactating dairy cows diagnosed with any degree of clinical mastitis treated with cefquinome (cefq) were included in the initial search. The following outcomes were selected for the analysis: clinical cure (CC), bacteriological cure (BC), somatic cell count (SCC), milk production, recurrence, and culling. Two reviewers did independent screening of the references, data extraction, and assessed the risk of bias (ROB) of included studies. A random effects MA using the Mantel-Haenszel method was conducted to compare extended therapy (ET) vs standard therapy (ST) in two trials. Results are shown as odds ratios and their confidence interval at 95%.

Results: Four RCT were included. Protocols compared were: (A) Intramammary (IMM) 75 mg every 12 h for 3 times (ST), (B) Protocol A plus intramuscular (IM) injection 625 mg at 0 and 24 h, (C) 75 mg IMM 3 infusions every 12 h plus 3 infusions every 24 h (ET), (D) 75 mg IMM every 12 h on d 1 and once a day for 4 d, and (E) Protocol D plus 1 mg/kg IM every 24 h for 5 times. Overall, ET compared to ST failed to show a benefit on CC, (OR: 1,0 [IC: 0,6-1,7]), BC (OR: 1,3 [IC: 0,9-1,9]), and reduction of quarter-level SCC to <200.000 cel/mL (OR: 1,4 [IC: 0,9-2,1]). After controlling by potential confounders, there was no evidence of improvement in BC (OR: 0,8 [IC: 0,4-1,3]) in a MA of the same two trials. Significant predictors for BC were: staphylococci infections (OR: 0,3 [IC: 0,1-0,9]), the interaction between duration of treatment and streptococci infections (ET over ST) (OR: 3,1 [IC: 1,1-8,9]), and SCC before treatment (OR: 0,4 [IC: 0,2-0,9]). The latter indicates that the higher SCC prior to treatment, the lower cure probability. One study found a significant difference in CC comparing A and C (OR: 3,0 [IC: 1,0-9,2]) by AD, where pretreatment cow udder firmness was a significant predictor of the outcome. Clinical persistence or recurrence was significantly lower when comparing protocols D (OR: 0,4 [IC: 0,1-0,5]) and E (OR: 0,3 [IC: 0,2-0,7]) to protocol A, after survival analysis conducted by the authors where the effect of the farm, quarter location, parity, and use of NSAIDs were significant predictors. Possible risks of selection, detection bias and performance bias were identified.

Conclusions: Results should be cautiously interpreted due to the ROB that might compromise validity. No difference was found in BC, CC or lowering the SCC in the overall MA. No differences in BC were found after controlling for confounders. Clinical cure following ET protocol was different in one study, showing a positive effect. The evidence supports the use of cefq to treat clinical mastitis caused by streptococci following an ET protocol; otherwise, ST should be the choice. Nevertheless, cow factors should be analyzed before treating the case to comply with a prudent use of antibiotics, such as cefq.

MD1-014-001

A randomized clinical trial to evaluate the effectiveness of incomplete milking in early lactation to reduce ketonemia and hyperketonemia

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Objectives: Hyperketonemia is a metabolic disorder of dairy cows. Generally, affected animals do not demonstrate clinical signs. It can cause lower milk production, poorer subsequent reproductive performance, predispose to serious illnesses and leads to early herd removal. In order to reduce ketonemia and hyperketonemia during the post-partum period, recent data have shown that limiting fat mobilization in fresh cows could be a relevant strategy. The objective of this study was to quantify the impact of a volume-controlled milking protocol (VCMP) on blood ketonemia and on the occurrence of hyperketonemia during the first 3 weeks of lactation.

Materials and Methods: Thirteen commercial dairy herds located within 100 kilometers of Saint-Hyacinthe (Quebec, Canada) were recruited to participate in a randomized clinical trial. From December 2013 to March 2015, 846 multiparous cows were randomly assigned to the conventional (complete) milking protocol of the farm (CMP) or the VCMP of 10-14 liters per day during the first five days in milk (DIM). A total of 427 and 410 cows were attributed to the CMP and VCMP groups; nine cows were lost to follow-up before start of the milking protocol. Blood β -hydroxybutyrate acid (BHBA) concentration was measured to quantify ketonemia, three times, at weekly interval on each cow between 1 and 25 DIM using a Precision Xtra hand held device (Abbott Diabetes Care, Alameda, USA). Hyperketonemia was defined as BHBA > 1.2 mmol/L. Time period was stratified as: 0-5 DIM (i.e. treatment period), 6-15 DIM (i.e. first 10 days following end of treatment), and 16-25 DIM (i.e. following 10 days). Effect of treatment on ketonemia was investigated using a linear mixed model with the natural logarithm of the BHBA measurement as the dependent variable and with treatment group assignment, time period, and interaction between time period and treatment group as fixed effects. Random effects at cow and herd level were added to adjust for the multiple measurements per cow and the multiple cows per herd. A generalized linear mixed model considering hyperketonemia as dependent variable, using a logit link, and the previously described fixed and random effects, was also developed to investigate effect of treatment on hyperketonemia.

Results: Mean blood BHBA concentrations during the 0-5, 6-15, and 16-25 DIM periods were 0.76 and 0.80, 0.96 and 1.10, and 1.22 and 1.16 mmol/L for the VCMP and CMP groups, respectively. A tendency for lower blood BHBA ($P < 0.08$) in treated cows was observed during the treatment period (i.e. 0-5 DIM), while lower concentrations were observed during the 6-15 DIM period ($P < 0.01$). Mean blood BHBA concentrations were similar between groups during the 16-25 DIM period ($P = 0.37$). The proportion of hyperketonemic cows during the 0-5, 6-15, and 16-25 DIM periods were 8.4 and 12.2, 23.5 and 28.5, and 32.7 and 30.5% for the VCMP and CMP groups respectively. Treatment was not associated with the occurrence of hyperketonemia during the 0-5 ($P = 0.13$), 6-15 ($P = 0.12$), and 16-25 DIM ($P = 0.54$) periods.

Conclusions: The evaluated milking protocol was an effective way to reduce ketonemia during the early postpartum period, but did not affect significantly odds of hyperketonemia. Further studies should

investigate the effect of VCMP on milk production, postpartum diseases, reproduction performance, udder health and culling.

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MD1-014-002

Survey of metabolic indicators in Irish periparturient cows ('transition period')

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Objectives: The transition cow period has been defined as the period from 3 weeks pre-calving until 3 weeks post-calving. This is a hugely important period characterised by significant changes in the endocrine status of the cow and the rapid mobilisation of somatic energy & protein reserves due to a reduction in feed intake when nutrient demand for the developing foetus and the upcoming lactation are increasing. The objective of this survey was to use metabolic profiles to monitor the management of cows in the transition period.

Materials and Methods: In the spring of 2015, Athlone and Kilkenny Regional Veterinary Laboratories conducted a transition cow survey within their catchment areas. Over 700 blood samples were received from batches of 6-12 cows in the transition period in 92 different herds. They were tested for the following metabolic parameters: magnesium, inorganic phosphorus, copper, urea, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB) and glutathione peroxidase (GSHPX). Calcium was measured in those cows that were within 48 hours of calving.

Results: Over 70% of these herds had at least one animal with serum magnesium concentrations below reference ranges: these animals are at risk of acute clinical hypomagnesaemia (grass tetany/ grass staggers). Low magnesium may also complicate cases of clinical hypocalcaemia (milk fever). 49% of these herds had at least one animal with serum urea concentrations below reference ranges which may indicate suboptimal protein intake. 50% of the herds with cows within 48 hours of calving ($n = 44$) had at least one of these cows with serum calcium below reference ranges. Two thirds of all herds had at least one animal with serum copper concentrations below reference ranges. Raised non-esterified fatty acid (NEFA) values (> 0.7 mmol/L) were identified in 27% of these herds or 18.6% of the samples tested. This metabolite is an index of negative energy balance and suggests that these animals were not consuming sufficient energy in their diets for the heavy metabolic requirements of late pregnancy & early lactation.

Conclusions: Blood biochemistry profiles are a useful tool in the monitoring and management of the transition cow and in investigating performance-related problems during this critical period. This study suggests that many Irish herds may have indicators of sub-optimal management of transition cows, and that a suite of metabolic indicators should be routinely monitored as an integral part of a comprehensive herd health plan. This survey work will be continued across more regional veterinary laboratories in spring 2016.

MD1-014-003

ACTH-test sensitivity affects disposition for high body condition in dairy cows during the transition period

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Objectives: In dry dairy cows feeding should be adjusted to requirements to avoid over-conditioning at calving because high body condition is a major risk factor for ketosis in early lactation. However, high body condition appears to have also a genetic and epigenetic background. The individual sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis and the release of cortisol as tested in the ACTH (adrenocorticotrophic hormone) test vary considerably between cows. Thus, aim of the study was to test the relationship between HPA-axis reactivity and body condition in dairy cows during the transition period.

Materials and Methods: In 22 pluriparous German HF Dairy cows, kept in free stalls with cubicles and fed a TMR ration based on grass and corn silage and concentrate, an ACTH challenge was performed on day(d)106 post partum (pp). Before and after injection of ACTH (80µg) cortisol concentrations were measured in blood samples taken in short term intervals from indwelling jugular vein catheters. For statistical evaluation baseline and peak concentrations and the area under the time-concentration curve (AUC) were assessed. By means of sonography subcutaneous (SCAT) and total abdominal adipose tissue mass (AbdomAT) were estimated at d-42, d1, d21 and d100 relative to parturition and their dynamic changes (in kg/d) were calculated separately for the dry and the lactation period. Cows were allocated to three groups, low (N=7), intermediate (N=8), and high (N=7) responder, according to their results in the ACTH test. For statistical evaluation ANOVA for repeated measurements were used with Tukey test for multiple comparisons of means (SAS package 9.3).

Results: Generally SCAT was about half as much as AbdomAT in studied cows ($p < 0.001$). At d-42, d1, d21 and d100 mean SCAT ($p < 0.01$) and AbdomAT ($p < 0.01$) were significantly lower in high compared to intermediate and low responder in the ACTH test. In average high responder in the ACTH test gained in the AbdomAT before parturition and mobilized after parturition from AbdomAT similar amounts as low and intermediate responder. Mean gain and mobilization of fat in SCAT was significantly less before and after parturition in high responder compared to low and intermediate ($p = 0.029$). However, relative to depot sizes of SCAT and AbdomAT mobilization of fat from each depot was not significantly different between groups.

Conclusions: According to results HPA axis sensitivity affect lipid metabolism in dairy cows during the transition period. High responder in the ACTH test are throughout the transition period leaner and mobilize less fat from SCAT after parturition which may affect disposition for subclinical ketosis in early lactation. Lipid metabolism of SCAT and AbdomAT appear to be differently affected by HPA axis sensitivity. The genetic and epigenetic background of HPA axis sensitivity need further evaluation in dairy cows.

MD1-014-004

Subclinical Hypocalcemia In French Dairy Cows: Study In A Veterinary Practice In The Department Of The Ardennes

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Objectives: As there is not any data on French dairy herds, a study was designed in order to determine if subclinical hypocalcemia also exists in France. For that purpose, calcemia around calving was measured in 106 Holstein cows from a veterinary practice in the Ardennes.

Materials and Methods: The prevalence of subclinical hypocalcemia, i.e. calcemia decrease at calving below 80 mg/L (or 85 mg/L depending on authors) without associated clinical signs, is not described to date in France. Nevertheless, this disease could have a lot of consequences for the dairy cow: it could represent an additional stress at calving, it could also increase early lactation negative energy balance, impair immune system, and make dairy cows more susceptible to postpartum health disorders. According to several American studies, subclinical hypocalcemia could affect up to 25% of primiparous cows and 50% of multiparous cows.

The study was carried out between August and November 2014. Eight different Prim'Holstein herds were included on a voluntary basis. Two herds were organic farms. In total, 106 cows could be recruited during the study period.

Calcemia was measured one week before calving, then 12 to 24 hours, 4 days and 8 days after calving, then each week if calcemia remained below 85 mg/L. Subclinical hypocalcemia threshold retained was 80 mg/L and a cow was considered in subclinical hypocalcemia if one blood sample was below 80 mg/L, without any hypocalcemia clinical sign.

Results: Among the 106 cows included in the study, 45% exhibited subclinical hypocalcemia, notably 16% of primiparous cows and 57% of multiparous cows. A significant association was evidenced between a low calcemia at calving and several postpartum diseases like metritis, mastitis, retained placenta and ketosis ($p = 0.03$ for retained placenta and $p = 0.01$ for at least one of these four diseases). A trend to develop at least one of these diseases was observed for cows with a calcemia under 83 mg/L ($p = 0.06$). A clear association between subclinical hypocalcemia and these diseases was observed from 75 mg/L of calcemia ($p = 0.03$). No significant association was emphasized between on one hand subclinical hypocalcemia and on the other hand body condition score, rumen fill score, lameness score, urinary pH (all those criteria being estimated during the week before calving), magnesemia (at calving), or β -hydroxybutyrate serum concentration (determined at 4 days and 8 days postpartum). However, hypocalcemic cows produced, on average, 4.8 kg more milk in the first month of lactation when compared with other cows ($p < 0.01$). No significant difference about milk quality was found: milk protein content, milk fat content and somatic cell count were not significantly different.

Conclusions: This study is to our knowledge the first that stresses the importance of subclinical hypocalcemia in France and that underlines the association with postpartum diseases in this context. Moreover, subclinical hypocalcemic cows produced on average more milk than control cows.

MD2-015-001

Prepartum plane of energy in dairy cattle: effects on production, energy balance, glucose tolerance, and adipose and muscle tissue insulin signaling

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Objectives: The transition from late gestation to early lactation poses tremendous metabolic challenges to dairy cows. Interest lies in providing optimal nutrition during the dry period to improve adaptation to lactation. Overfeeding energy to prepartum dairy cows has been associated with detrimental effects in early lactation. The study objectives were to assess the effect of dry period nutritional strategies varying in energy content on energy balance, milk production, glucose tolerance, and adipose and muscle tissue insulin signaling.

Materials and Methods: Multiparous dairy cows (n=84) were enrolled at dry-off (57 d before expected parturition) in one of three different ad libitum TMR dietary dry period nutritional groups: those fed a controlled energy diet during the entire dry period, formulated to supply 100% of predicted requirements (group C); those overfed energy by 50% of predicted requirements (group H); and those that received the same TMR as group C during the far-off period and then a diet supplying energy in excess of 25% of energy requirements during the close-up period only (group I). Diets were balanced for metabolizable protein and all cows were fed the same fresh cow TMR until the end of the study at 42 DIM. Intravenous glucose tolerance tests (IVGTT) with rapid infusion of 0.25 g/kg of glucose were performed at 28 and 10 days before expected parturition, as well as 4 and 21 days after calving in all cows. Backfat thickness and diameter of M. longissimus dorsi were measured by ultrasonography at six times throughout the study. Subcutaneous adipose and external oblique muscle tissue biopsies were taken at the same time-points immediately before and 60 min after glucose infusion. All IVGTT samples were analyzed for insulin, glucose and NEFA concentrations; baseline samples were analyzed for β -hydroxybutyrate (BHB) and glucagon concentrations. Adipose and muscle tissue protein were extracted from 12 cows each in group C and H, and subjected to immunoblotting, for phosphorylated and total AKT and ERK. Statistical analysis was performed using SAS 9.3 adjusting for multiple comparisons using Tukey's posthoc test.

Results: Compared with cows fed a controlled energy diet prepartum, cows overfed energy during the dry period showed higher concentrations of BHB (0.84 vs. 0.61 mmol/L, $P = 0.04$) and NEFA (0.80 vs. 0.66 mmol/L, $P = 0.02$) postpartum whereas milk production in early lactation was unaffected ($P = 0.98$). Of all cows, 25, 36 and 43 % in groups C, I and H had at least one episode of BHB over the cut-off of 1.2 mmol/L (=positive test). The overall median time to first positive test was 7 days. Within the first 21 DIM, cows in group C, I and H had 13, 32 and 31 test positive episodes out of 250 tests in each group ($P = 0.004$). Dry matter intake (DMI) and energy balance prepartum were higher in cows fed higher energy diets ($P < 0.001$), but DMI did not differ postpartum between treatment groups ($P = 0.99$) although energy balance tended to be less negative postpartum in the controlled energy group ($P = 0.09$). Glucose tolerance and insulin response to a glucose bolus did not differ in the three groups ($P > 0.10$), but baseline concentrations of glucose and insulin 4 days postpartum were lower in cows overfed energy during the whole duration of the dry period ($P \leq 0.09$). Insulin signaling at baseline and in response to an endogenous insulin stimulus measured as the

ratio of phosphorylated to total AKT and ERK did not differ in adipose and muscle tissue biopsies from group C and H.

Conclusions: Overfeeding energy in the prepartum period was associated with increased concentrations of markers of negative energy balance postpartum despite the absence of differences in milk production. Overfeeding in the dry period did not affect insulin sensitivity postpartum in our study. Lower resting glucose concentrations postpartum in the overfed group can be interpreted either as a decreased gluconeogenic capacity or a decreased uptake of glucose in the periphery, however hepatic gluconeogenesis in this study was not determined.

MD2-015-002

A field study about incidence, risk factors, and consequences of ketosis in dairy cattle

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Objectives: Dairy cows in early lactation must transition from the demands of late gestation to those of early lactation. Cows failing to adapt commonly incur an elevation of circulating ketone bodies in blood. This condition is known as ketosis.

The objectives of this study were to first determine the incidence of ketosis in different areas and production systems in Spain, and second to identify potential predisposing factors for ketosis and the consequences of this affliction on milk production.

Materials and Methods: Nineteen dairy herds distributed in four distinct areas of Spain (North: 8 herds and 239 cows; Northeast: 5 herds and 1,537 cows; South: 3 herds and 625 cows; and Northwest: 3 herds and 694 cows) were enrolled in this study for a period of 13 months. Average milk production of the 19 herds was 33.4 kg/d. Each herd was visited at least once a week by a veterinarian who collected data about cow performance and disease. All participant veterinarians held a meeting before the initiation of the study to reach a consensus about the criteria for diagnosing retained placenta, metritis, hypocalcemia, and mastitis. Also, each veterinarian collected a blood sample by venipuncture of the coccygeal vessels to determine blood β -OH-butyrate (BHB) concentrations using a cow-side system (Precision Xtra meter, Abbott Laboratories, Abbott Park, IL) between -14 and -7, 1 and 7, and 14 and 21 d relative to calving. At these time points, cows were also evaluated for body condition score (BCS) with scale from 1 to 5. Cows were classified as having subclinical ketosis (SCK) whenever blood BHB was between 1.2 and 3.0 mM, and classified as having clinical ketosis (CK) when blood BHB was ≥ 3.0 mM.

Results: Incidence of ketosis (blood BHB ≥ 1.2 mM) was 28.3%.

Incidence of SCK was greatest in summer and lowest in fall, whereas the incidence of CK was greatest in both spring and summer. Cows that calved in summer had 1.4-fold greater odds ($P < 0.01$) and those that calved in fall had 0.77-fold lesser ($P < 0.01$) odds of having ketosis than those that calved in winter. The odds of having ketosis was 2.25-fold greater ($P < 0.001$) for cows with twins than with cows that had regular calvings. Multiparous cows had 2.34 greater ($P < 0.001$) odds of incurring ketosis than primiparous cows. Furthermore, for every unit (a 'unit' is '0.25') increase in BCS before calving cows had a 2.1-fold increase ($P < 0.001$) in the risk of undergoing ketosis, and for every unit

of BCS loss between 1 week before and 3 weeks after calving, the risk of ketosis increased ($P < 0.001$) 1.96-fold. Multiparous cows producing more milk in the previous lactation were more ($P = 0.04$) likely to develop ketosis than their lower-producing herdmates. Ketosis prevalence was 30.9, 31.1 and 34.2% in cows producing less than 10,800, 10,800-12,700 and 12700-14000 kg in their previous lactation, respectively; and reached 39.7% in cows that produced over 14,000 kg of milk in their previous lactation.

Lastly, cows that incurred ketosis produced more ($P < 0.01$) milk (37.9 ± 1.83 kg/d) during the first 15 weeks of lactation than those that did not (35.4 ± 1.83 kg/d). Cows that had ketosis produced the same amount of milk the first 4 weeks after calving, but they produced more milk than those that did not have ketosis thereafter.

Conclusions: The overall incidence of ketosis was about 28%. Multiparous cows, cows carrying twins, and cows calving in summer have a greater risk of incurring ketosis. And as the difference in BCS between 1 week before and 3 weeks after calving increases the risk of incurring ketosis also increases. Multiparous cows producing more milk in the previous lactation are more likely to develop ketosis in their subsequent lactation than their lower-producing herdmates. Cows that experience ketosis produce the same amount of milk the first 4 weeks of lactation, but then they produce more milk than those that did not have ketosis.

MD2-015-003

Is subclinical hypocalcemia a truly subclinical disorder?

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Objectives: Subclinical hypocalcemia (SCHCa) presents no symptoms related to low serum Ca concentration (< 8.3 mg/dL); cows not recumbent, not showing excitability, nervousness, weight shifting and hind feet shuffling. Given the lack of information regarding clinical examination findings of SCHCa cows, the objective of the study was to investigate any possible association among them.

Materials and Methods: The study was conducted in 8 commercial dairy herds located in Northern Greece, with 770 Holstein cows. Each cow was clinically examined and blood sampled on the 1st and 2nd day after calving. Rectal temperature (RT), heart rate (HR) respiratory rate (RR), number of ruminal contractions per 2 minutes (RC) and rumen fill score (RS) were recorded. Serum Ca concentration was determined with atomic absorption spectrophotometry. Health status was monitored during the first 8 days after calving; cows that presented metritis, mastitis, ketosis or left displaced abomasum were excluded from the study. The final data set included 420 day 1 and 427 day 2 complete cow records, respectively. Clinical examination findings potentially associated with SCHCa were: a) $RT < 38.5$ C, b) $HR > 100$ beats per minute, c) $RR > 50$ breaths per minute, d) $RC < 2$ per 120" and e) $RS < 3$ (5-point scale; 1: empty rumen, 5: well-filled rumen). Their relationship with SCHCa was examined: i) singly, ii) all pair combinations with 1 or both being present, iii) all triple combinations with 2 or 3 being present, iv) all quadruple combinations with ≥ 2 and ≥ 3 being present, and v) all 5 together, with ≥ 2 and ≥ 3 being present. Presence of findings for different SCHCa severity levels was investigated using 3 serum Ca thresholds;

< 8.3 mg/dL (A), < 8.0 mg/dL (B) and < 7.5 mg/dL (C). Sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV and NPV, respectively), and 95% confidence intervals (95% CI) were calculated for all clinical examination findings and SCHCa thresholds combinations.

Results: Prevalence of SCHCa during day 1 was 36.9%, 24.8% and 13.1% for thresholds A, B and C, respectively; that of day 2 was 36.5%, 28.1% and 16.4%, respectively. Se and Sp values were very similar across SCHCa thresholds. Mean Se of all combinations of clinical findings was 0.24 and 0.21, for days 1 and 2 respectively. However, for RS – HR, RS – RR and RS – RT, it ranged from 0.81 to 0.89; Sp was low in these cases: 0.23 and 0.21 for days 1 and 2, respectively. Mean Sp of all combinations of clinical findings was 0.77 for both days 1 and 2. When RC was combined with HR, RR and RT, as pairs or triples with all findings present or when they were all four combined with ≥ 3 findings present, Sp was > 0.90 . Generally, clinical findings combinations had either low Se (< 0.25) - high Sp (> 0.75), high Se (0.80-0.85) - low Sp (< 0.25) or both moderate, ranging from 0.45 to 0.55. Mean PPV differed across SCHCa thresholds and was usually low, 0.39 and 0.36 for threshold A, 0.29 and 0.28 for B, and 0.14 and 0.20 for C, on days 1 and 2, respectively. On day 1, a PPV of 0.67 resulted when RC, RR and RT were combined and all three findings were present (thresholds A and B); 95% CI was 9.4% to 99.2%. On day 2, a PPV of 0.67 resulted when: a) RC and HR were combined and both findings were present (thresholds A, B and C); 95% CI was 22.3% to 95.7%, and b) RC, RR and HR were combined and all three findings were present (thresholds A, B and C); 95% CI was 9.4% to 99.2%. Mean NPV was 0.63 and 0.64 for threshold A, 0.76 and 0.72 for B, and 0.87 and 0.84 for C, on days 1 and 2, respectively.

Conclusions: When present, certain clinical examination findings may be indicative of SCHCa. On day 2, the probability a cow with none or only one rumen contraction per 120" and tachycardia (heart rate > 100) to be subclinically hypocalcemic is 67%. The calculated wide 95% CI range limits the practical application of this finding. More research is warranted towards cowside detection of SCHCa without blood testing.

MD2-015-004

Prevalence of subclinical hypocalcaemia in spring-calving pasture-based dairy herds in New Zealand

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Objectives: Prevalence of subclinical hypocalcaemia in dairy cows is not well documented in New Zealand. The aim of this study was to estimate the true herd-level prevalence of subclinical hypocalcaemia as measured by serum calcium levels of < 2.0 mmol/L in Waikato dairy herds.

Materials and Methods: Clinically normal mature cows (i.e. ≥ 2 nd lactation; average $n=14.2$ per herd) who were within 48 hours of calving were sampled on one or two calendar days between the 10th July 2015 and 11th August 2015, from 74 spring-calving pasture-based dairy herds from the Waikato region of New Zealand.

Cows were excluded if they had had any antibiotic or anti-inflammatory treatments in the previous 14 days or if they had been diagnosed with any clinical disease around calving.

Cow level data (age, breed, calving date, sampling date and body condition score (on a 1-10 scale) were recorded and a blood sample was collected from the coccygeal vein. Serum samples were analysed for total calcium concentration within 48h of collection by a commercial

veterinary laboratory (New Zealand Veterinary Pathology, Hamilton, New Zealand).

Data were entered into a Microsoft Access database and descriptive statistics performed used StataSE 12.

Results: The average herd size was 467 (range = 160-1100) cows.

The average age of the sampled cows was 5.2 (range 3-13) years, the average BCS was 4.7 (range 3.5-6.5) and the predominant breeds were New Zealand Friesian (44%), Kiwicross (38%) and Jersey (11%).

Cows were sampled on the day of calving (33%), within 24 hours of calving (49%) or within 48 hours of calving (17%).

The average serum calcium concentration for the 1,071 samples was 2.07 mmol/L (SD=0.31 ; Range = 0.99-2.85) and 33% (31-36 95%CI) of animals had concentrations <2mmol/L. The median herd prevalence of subclinical hypocalcaemia was 33% (20-46% IQR).

The prevalence of subclinical hypocalcaemia decreased with increasing time from calving with 46% affected on day 0, 28% on day 1 and 20% on day 2. However, time from calving to sampling was correlated with herd.

Conclusions: This observational study has identified that subclinical hypocalcaemia is highly prevalent in spring-calving pasture-based dairy herds in New Zealand. There is large between-herd variation in the prevalence of this condition. Prevalence of subclinical hypocalcaemia declines with time postpartum. Further work is required to define factors associated with the between-herd variation in this condition.

MD2-015-005

Interaction between individual adrenal cortex sensitivity and insulin sensitivity in German Holsteins

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Objectives: Subclinical ketosis mainly caused by negative energy balance and subsequent lipomobilisation is a frequent health disorder in dairy cows after parturition. Reduced insulin sensitivity commonly is seen as a promoting factor for excessive lipomobilisation. Furthermore, cows show considerable differences in their individual adrenal cortex sensitivity, leading to varying plasma cortisol levels as a reaction to stress. Cortisol, however, may contribute to decreased insulin sensitivity. The aim of this study was to evaluate the correlation between the individual adrenal cortex sensitivity and insulin sensitivity in dairy cows.

Materials and Methods: In our study an intravenous glucose tolerance test (GTT; 0.25 g glucose / kg bodyweight) followed by an insulin challenge (IC; 1 µg insulin / kg bodyweight) was performed on 20 multiparous German Holstein cows on day 106 postpartum. Blood was collected after glucose and insulin injection for 110 minutes in short term intervals to measure glucose and insulin concentrations. Three days later an ACTH (adrenocorticotrophic hormone) test was performed by injecting exogenous ACTH (80 µg / cow) and blood samples were taken for 120 minutes in short term intervals to assess cortisol levels. Furthermore, weekly from 42 days ante- until 106 days postpartum blood samples were collected from all cows for analysis of insulin, glucose and fatty acids (NEFA) to calculate surrogate indices of insulin sensitivity,

as follows: Quantitative insulin sensitivity check index (QUICKI) = (1/[log(Glucose (mg/dl)) + log(Insulin (µU/ml))]) and revised quantitative insulin sensitivity check index (RQUICKI) = (1/[log(Glucose (mg/dl)) + log(Insulin (µU/ml)) + log(NEFA(mmol/l))]). For statistical analysis base values, peak, nadir and area under the curve (AUC) were determined for ACTH test (cortisol), GTT (glucose, insulin), and IC (glucose, insulin). Also glucose elimination rate was calculated from GTT data. Results of the ACTH test were correlated (SPEARMAN) with results of the GTT and IC as well as with surrogate insulin sensitivity indices by means of the SAS computer package (Vers. 9.3).

Results: On day 106 after parturition results of the ACTH test (base value, peak, and AUC) revealed no significant (p > 0.05) correlations with QUICKI and RQUICKI. Base cortisol concentrations of the ACTH test correlated significantly with AUC glucose GTT (r = -0.45, p = 0.047) and nadir glucose IC (r = -0.49, p = 0.030) and peak ACTH test with nadir glucose IC (r = -0.51, p = 0.021). Peak and AUC cortisol ACTH correlated significantly with RQUICKI on d-7 (r=0.65, p = 0.002; r=0.46, p = 0.041, resp.) but not with QUICKI.

Conclusions: According to results individual adrenal cortex sensitivity appears to have only marginal effects on insulin sensitivity in dairy cows as measured by glucose tolerance test, insulin challenge and surrogate insulin sensitivity indices.

MD2-015-006

Periparturient immunosuppression and strategies to improve dairy cow health during the periparturient period

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Objectives: Selection for production traits with little or no emphasis on health and fitness traits has led to a higher incidence of disease in dairy cattle, including mastitis which is unfavourably genetically correlated with production traits. This increase in disease incidence is especially evident during the periparturient period when a temporary impairment in immune function (immunosuppression) occurs. In this review we aim to explore the causes of periparturient immunosuppression and propose short and long-term strategies aimed at improving dairy cow health and welfare during this critical period.

Materials and Methods: A systematic review on the causes of periparturient immunosuppression in dairy cattle coupled with their control strategies was reviewed based on literature search from 1980's to 2015. The methodology used was based on the protocol outlined by Sargeant et al., 2006. This involved specifying the research questions, a decision on the review process, criteria for the inclusion and exclusion of studies/literature, assessment of study quality, data extraction and synthesise. Literature was searched using the library discovery search engines of The University of Melbourne, University of Guelph and CSIRO. "PubMed" and "Google Scholar" searches were also used.

Results: A high proportion of cows experience immunosuppression during the periparturient period as a consequence of the physiological

demands of parturition, lactogenesis and colostragenesis. A focus on proper energy and nutritional balances during the periparturient period of dairy cows are important short-term strategies to improve the health and welfare of the transitioning cow. Research focusing on the arrest and reversal of the increased incidences of production-related health problems in the current modern dairy cow are incorporating immune responsiveness measures in selection and breeding programs. The development of methods to assess the immune competence phenotype of dairy cows has facilitated the adoption of selection and breeding programs aimed at improving general disease resistance of dairy herds. This approach has the potential to identify cows that are better able to cope with the challenges posed by their production environment whilst incorporating other health and survival traits. Immune response phenotyping, involves identification and selection of animals with an enhanced general immune responsiveness, as assessed by combining measures of an animal's ability to mount both antibody- and cell-mediated adaptive immune responses.

Conclusions: We propose a longer-term strategy based on improving the resilience of dairy herds through the selection of animals with enhanced resilience, and as a consequence, are better able to cope with the environmental challenges posed by their production environment.

MD2-015-007

Evaluation of sampling strategies for reticuloruminal pH measurements in individual cattle for the assessment of herd status

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Objectives: Single time point or discontinuous measurement of reticuloruminal fluid pH has been used as a diagnostic tool to identify sub-acute rumen acidosis in lactating dairy cows, which is usually defined as 3 animals of 12 sampled with pH \leq 5.5. It is recommended that samples should be collected within 2-7 h after feeding. Our first objective was to use data from many intraruminal continuous pH monitoring boluses to determine the optimal sampling methodology. The second objective was to describe the variation in precision of estimation of pH values in a herd with the deployment of varying numbers of continuous pH monitoring boluses.

Materials and Methods: Continuously monitored reticuloruminal pH observations were obtained from devices deployed in clinically normal cattle on 13 German dairy farms and one British beef production research unit. Firstly, a single pH observation from within 15 minutes of a specified time of day was randomly sampled from each animal, ignoring the date on which the sample was taken. Then, a number of these animals were randomly sampled (without replacement) from the same farm, and the number of observations below the specified threshold was determined. A total of 10,000 iterations was used to give a probability estimate, and the whole process was repeated for each set of time of day, farm, threshold in the set {pH 5.0, pH 5.5, pH 5.8, pH 6.0}, number of sampled animals in the set {3, 4, 6, 12} and proportion of animals required to be below the threshold in the set {3/12, 1/3, 1/2}. To estimate the effect of number of

devices deployed for estimation of a herd's reticuloruminal pH status on the accuracy of the estimation, we used the continuous pH observations from the four farms with \geq 12 devices. A set of pH measurements taken from animals in the same herd at the same time and date was sampled with replacement from the available measurements. The absolute difference between the mean of the sample and the true mean of the observed pH from all animals at that date and time in that herd was recorded. A total of 1,000 iterations was used to give a mean absolute error for each combination of time of day, herd, and number of animals sampled.

Results: Reticuloruminal pH information was used from 111 cows in 16 herds, resulting in a total of 815,475 observations. The effect of herd (confounded by monitoring system) was highly significant ($p < 0.001$), with mean values being lowest in the British beef production unit during each time period.

There was a strong effect of time of day on the probability of detection of animals with pH values below specified thresholds, the probability of detection being consistently lower between 06:00 h and 14:00 h and higher between 18:00 h and 02:00 h. The pattern of diurnal rhythmicity differed among farms, being best described by a non-linear model with a single, double or triple sine-wave, broadly in line with frequency of offer of fresh feed.

To apply the accepted criterion of SARA of 3 from 12 animals sampled with pH \leq 5.5, for the beef herd on the transition diet, there was close to 100% probability that 3/12 animals would have a pH of equal to or below 5.5 between 18:00 h to 02:00 h, dropping to almost zero at midday.

The mean pH from a sample of 3 animals from herd A was within 0.1 pH units of the true mean 50% of the time, and within 0.25 pH units 99% of the time. In contrast the mean of a sample of 3 animals from herd N.3 was only within 0.25 pH units 50% of the time, and was within 0.8 pH units 99% of the time. Hence, the requisite number of boluses to be used for real-time monitoring purposes in any herd is dependent on both the inherent volatility in the herd as well as the desired level of accuracy.

Conclusions: Our study suggests that the currently accepted recommendation for the detection of SARA, as defined by a herd or group within a herd from which 3 of 12 randomly selected cows have a reticuloruminal pH (either by rumenocentesis or indwelling pH bolus) with a pH \leq 5.5, taken at a specified range of times after feeding is likely to be misleading in many instances, sensitivity being higher during the evening than daytime. The number of boluses required to provide an accurate representation of the true pH status of a herd varies with herd. Under stable nutritional conditions, as few as 3 boluses can provide an acceptable indication of herd status.

NHP-041-001

Surveillance 2014: A New Post Mortem Examination Service in the UKIngrid Den Uijl¹,*Jimmy Wiseman¹Roberto LaRagione¹Alasdair Cook¹¹University of Surrey, Guildford, United Kingdom

Objectives: In response to a review of animal health surveillance in England and Wales, a new system has been initiated that involves partnership between government, universities and private veterinary practices. The overall purpose is to provide early warning surveillance to identify new or re-emerging threats to animal and public health. The University of Surrey (UoS) is participating in this system by delivering a new model to perform surveillance post mortem examinations (PMEs) for several areas in England.

Materials and Methods: UoS in association with private veterinary surgeons, has recently started providing a subsidised surveillance PME service for the major farmed livestock species on behalf of Animal and Plant Health Agency (APHA). The service is delivered by UoS trained veterinary staff. The majority of PMEs will be conducted at new PME facilities at selected fallen stock sites around the country. The service commenced operation in February 2015 at Cowfold, West Sussex, and since then further sites have become operational in; Flagg, Derbyshire; Davidstow, Cornwall; Sutton Bonington, Leicestershire and Braintree, Essex.

All data and samples from these submission are managed by UoS. Farm and animal details as well as clinical history are collected and PME details are recorded in a purpose build digital system, PathPal™. Data collected from the PathPal™ system is exported to APHA to be merged for analysis of the national herd. The PathPal™ data is also analysed by the UoS. Maps are created to analyse the spread of disease, additionally an overview of the diagnoses made at PME are presented.

Results: During March-November 2015, 112 PMEs were submitted of which 61 were cattle, 31 sheep, eight birds, five pigs, three camelids, three goats and one deer. Some submissions consisted of multiple carcasses. The submissions were similar across all sites, with exception of Braintree, Essex, where submissions were lower than in the rest of the UoS allocated area.

In most cases a diagnosis was reached. Only 29% had a diagnosis not reached, however disease was localised to a particular body system in all but 4% of cases. Digestive, systemic and respiratory diseases made up 50% of our submitted caseload with circulatory, musculoskeletal and nervous disorders making up a further 30%, the remaining 20% were reproductive disorders.

Colisepticaemia was the most common diagnosis in neonates. In young cattle and sheep pneumonia was the most common diagnosis. Hepatic abscessation was the most common diagnosis in adult cattle. In adults sheep clostridial disease was often seen.

Conclusions: The study showed the possibility to carry out surveillance using PME facilities at fallen stock sites. In most cases a diagnosis was reached and some common diagnoses were presented. Further research using the automated data capture system will look into risk factors for common diagnoses and syndromic surveillance based on the collected data.

NHP-041-002

The Development Of 'Healthy Hooves', The Australian National Lameness Prevention ProgramJo Coombe^{1,2},*Erika Oakes²¹Faculty of Veterinary and Agricultural Sciences, University of Melbourne, ²Dairy Australia, Melbourne, Australia

Objectives: Dairy Australia is the national services body for dairy farmers and the industry in Australia. Unlike many other countries, Australia does not yet have a coordinated industry program to address lameness in dairy cows. In 2012, it became one of the key areas to be addressed by Dairy Australia. The objectives of "Healthy Hooves" are to promote animal welfare, industry sustainability and farm profitability through: a) establishment and adoption of a national lameness recording system, and b) improved management and prevention of lameness through the delivery of resources and training to farmers and their advisers.

Materials and Methods: The concept of a national program was developed in 2012 by bringing together many of the Australian key researchers, veterinarians, hoof trimmers and farmers for a stakeholder workshop. Since then, through the work of several steering groups, the framework of the 'Healthy Hooves' program has been put in place, with the target of a June 2016 launch. Key activities to achieve this target included 1) Resource development, including: a lameness investigation pack (aimed at advisors) and farmer resources (including lameness prevention guidelines, wall charts and booklets for lesion identification, data collection sheets and Standard Operating Procedures). 2) Updating of the farmer lameness workshop and development of webinars to upskill veterinarians or other industry trainers. 3) Video resources (a suite of videos that can be used for training and displayed on the Dairy Australia website). 4) Data activities (the development of a data capture tool) Performance of a full environmental scan of data capture in Australia and develop a platform that will allow farmers to capture lameness data quickly and easily, with agreement on an Australian industry approach to locomotion scoring.

Results: The resource development was completed as planned. The lameness prevention manual was written with the input of experts in the field, and will be edited for final publication. The lameness investigation pack is being piloted by several veterinarians prior to the launch of the program. Video resources were recorded with the input of a lameness expert from New Zealand, and the data capture tool is being developed with locomotion scoring based on the Dairy New Zealand 0-3 scale (0 being not lame, 3 being severely lame). Additionally the recording tool will have the ability to capture the most common lesions in the Australian pasture-based system. Webinars will be recorded by the launch date.

Conclusions: It is anticipated that following the launch of the Healthy Hooves project, there will be an increased awareness of lameness and farmers will have access to high quality resources to help them better manage it, with better methods to capture incidence. Additionally, it is hoped that using best practice guidelines, the development of an advisor base in Australia will enable farmers to access professional advice to manage the problem.

NHP-041-003

Quantifying the importance of Trojan dams in the national BVD eradication programme in IrelandFiona Reardon^{1,2},*David Graham³Tracy Clegg²Jamie Tratalos²Padraig O'Sullivan⁴Simon More²

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Objectives: A national eradication programme for bovine viral diarrhoea virus (BVDV) began in Ireland in 2013. To date, while Trojan dams are recognised as disseminators of infection, there is no published work quantifying how many persistently infected calves are born in herds due to the introduction of Trojan dams. Therefore, the objective of the current study was to quantify the importance of Trojan dams in relation both to the number of PI births directly attributable to them and the subset of herds where BVD introduction can be attributed to a Trojan dam.

Materials and Methods: The initial study population included all BVD+ calves born in Ireland in 2013 and 2014. Their dams were assigned a Trojan status based on birth date of the calf relative to the date that the dam entered the herd. A dam was considered to be a Possible Trojan if she entered the birth herd between days 30-120 of gestation (162-252 days before calving). For these animals, it was considered possible that infection had been contracted in either the birth herd or prior to entry. A dam was considered to be a Definite Trojan if she entered the birth herd after day 120 of gestation (less than 162 days before calving), having contracted infection prior to entering the birth herd. Herds with PI births were analysed by enterprise type, location, herd size and number of PI births. Dams of PI calves were analysed by age, parity, import status and number of movements during pregnancy. Source herd(s) were analysed by location, herd size and enterprise type. Data analyses were conducted using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and STATA 14 (StataCorp LP, College Station, TX, USA).

Results: A total of 23,976 known PI calves were born in 11,960 herds in Ireland during 2013 and 2014. The percentage of PI births attributable to Trojan dams was relatively stable across regions, ranging from 7.5% of PI births in the South-West to 11.2% in the Midlands. A Possible or Definite Trojan dam was present on 10.5% of herds with at least one PI birth in 2013 and 2014.

The percentage of PI births attributable to Trojan dams was highest in dual purpose herds, accounting for 12.5% of all PIs. Beef herds had the highest absolute number of Trojans at 1,178, representing 10.8% of beef PI births.

40% of all dams of PI animals were first calvers. Of these first calvers 15% were Trojans, dropping to 6% in second parity dams. 19% of PI births to Trojan dams occurred where the dam had moved back into their herd of origin. In 71% of herds that had a Trojan birth, this represented the only PI calf born on that farm in that year.

Movement records were analysed of 1775 Possible and Definite Trojan dams. 46.7% of dams had only one movement, which was a direct farm to farm movement. 40.5% had at least one movement through a mart, making it difficult to categorically trace the source of infection.

Conclusions: This research highlights the contribution of Trojan dams to the birth of PI calves in Ireland. The high proportion (71%) of instances where the only PI birth on a farm in that year could be attributed to a Trojan dam highlights their role in the spread of infection. While testing of calves within days of birth allows the prompt identification of PI calves born to Trojan dams, these results confirm the need to address the issue on a systematic basis. Further research is underway to evaluate the effectiveness of potential control methods in minimizing Trojan movements.

NHP-041-004

A Novel National Animal Health And Welfare Programme For Dairy Farms

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Objectives: The New Zealand dairy industry is the single most important export industry in New Zealand and by far the most economically important primary industry. However, it has lacked any form of national programmes incorporating disease monitoring or welfare assessment.

The Welfarm™ programme was developed to provide a national programme which monitors and benchmarks animal health, production and welfare, and which is of value to both the producer and to other stakeholders.

This presentation discusses the outcomes and challenges in a voluntary user-pays welfare based scheme.

Materials and Methods: The programme was developed by XLVets™ in New Zealand. A regional pilot programme was used as the basis for broadening into a national project. The project is now in its third year. Data is captured by veterinarians, from farmers themselves, through customer sales software, and via other electronic platforms, and is consolidated into a single online, benchmark dashboard.

The online data is updated in real time and provides vets and farmers with a view of their farm(s) with the regional and national average. The online platform has been developed to accommodate a large number of farms and measurements.

Data are collected from a range of areas which cover animal health, welfare and productivity. Therefore, body condition scoring (BCS), locomotion scoring and tail scoring are carried out. BCS is performed 4 times annually. Milk quality is benchmarked via bulk milk SCC, number of mastitis treatments and incidence of adverse milk grades. Reproductive data including non-pregnant rate and 6wk in-calf rate (ICR) are gathered. Other indices including mortality, calves reared, NSAID use, number of vet visits and antimicrobial use are also recorded and benchmarked.

Results: During the 2014/2015 season, data were collected via XLVets™ member clinics from 84,325 cows on over 150 farms nationally.

The mean annual mortality rate was 3.2% and the mean replacement rate was 26.6%. Mean herd size (cows wintered) was 613. The mean number of vet visits per year per 100 cows was 7.2, with a wide variation from 3- 9.8. This number was associated with herd size, such that the larger sized herds tended to have lower vet visits/100 cows.

Body condition showed a strong seasonal variation which accords with previous industry data. However, a regional variation was also detected, which may accord with feed quality and quantity in a largely pasture-based dairy system.

The largest proportion of antibiotic use was associated with dry cow therapy when measured using an animal daily dose (ADD) methodology but this was not the case when population corrected units (PCU) was used.

Conclusions: Although veterinarians and animal health experts recognise the value of maintaining efficient and appropriate welfare standards, one of the challenges with voluntary quality assurance programmes in a user-pays environment is developing value for the user.

The Welfarm™ programme has started the process of benchmarking animal health and welfare within New Zealand, and demonstrated that, for many indices, industry results are not necessarily where they would

be expected. It has provided the industry with quality benchmark data which can now be broadened into the further development of a national welfare programme.

NHP-041-005

Comparative assessment of surveillance programs to proof freedom of BHV1 in dairy herds in a disease-free and endemic situation.

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Objectives: This study aimed at assessing the epidemiological performance of two surveillance systems for detecting bovine herpesvirus type 1 (BHV1) infection in dairy herds in the Netherlands as well as monitoring freedom from disease at herd level.

Materials and Methods: EU Decision 2004/558/EC and 2007/584/EC describe the requirements for cattle holdings to attain and maintain an official free BHV1-free status, which is the current basis for a country to obtain the Article 10 status as laid down in EU Directive 64/432/EC. Briefly, for dairy herds the requirements are (i) a test-and-cull procedure for all animals >9 months to obtain a free status, and (ii) yearly serology of all lactating cattle to maintain the free status. For this study, we designed an alternative surveillance system based on the voluntary BHV1 control system in the Netherlands. Basically, the program is based on (i) a test-and-cull procedure for all animals >12 months to attain the free status, (ii) monthly bulk milk testing to maintain the free status, and (iii) risk-based testing of purchased animals that originate from non-free herds. A scenario-tree analysis method was implemented to determine the sensitivity of the conventional (EU) and alternative (NL) surveillance system. To do so, the sensitivity of each surveillance system component for detecting a BHV1-infection at herd level (with an animal-level design prevalence of 10%) was calculated. Also, the probability of freedom at herd-level was estimated in time-steps with a 1-month interval, adjusted for the risk of introduction of BHV1 between tests. Analyses were carried out for a disease-free and an endemic country-level situation.

Results: Both systems reached a 100% sensitivity of the 'obtaining free status' component of the program. The sensitivity of the 'monitoring free status' component to classify a herd as positive when infected at an animal-level design prevalence of 10% was 100% for the conventional (EU) program and 96% for the alternative (NL) program. In an endemic situation, the EU program led to a probability of freedom of 99.6 - 100%, depending to the number of months since the yearly serological investigation of all lactating cattle. The alternative program led to a probability of freedom of 99.96 in months when no animals were purchased and 99.88% when animals from non-free herds were purchased. In a situation where the country is free from BHV1, both scenarios will lead to a constant probability of freedom of 99.9% or more. Additional and more detailed results will be presented at the conference.

Conclusions: This study presents the results of an epidemiological methodology that could be applied to compare surveillance strategies to proof disease freedom. The assessment of various surveillance designs can be highly useful to support decision-making towards a more risk-based approach of animal health surveillance.

NU-016-001

Inter-observer agreements on body condition scores and on the difference in body condition scores in dairy cows

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Objectives: The correlation between body condition score (BCS) and energy reserves, the ease and rapidity of scoring, and the relatively high intra and inter-observers repeatability make it a widely used herd management tool in bovine practice and in scientific studies. However, difference (loss or gain) in measurements of BCS (DMBCS) is generally more relevant when monitoring dairy cows. It remains unclear if DMBCS for a group of cows by several observers would provide a good agreement. Therefore, the objective of this study was to investigate the inter-observer agreements on BCS and on DMBCS in dairy cows.

Materials and Methods: A total of 73 Holstein cows from one commercial herd were conveniently enrolled in this observational study conducted between April and September 2015. After a short revision of the Ferguson BCS scale, three observers independently assessed BCS between 1 and 20 days in milk (early lactation; exam-1) and again between 41 and 60 days in milk (peak of milk production; exam-2). Each observer measured all cows at exam-1 and exam-2. At the second exam, observers were blinded to the BCS reported at first exam. Inter-observer agreement on BCS at exam-1 and exam-2 was computed using a quadratic weighted Kappa statistic (κ). For each observer, DMBCS between exam-1 and exam-2 were calculated. Using κ , inter-observer agreement on DMBCS was also determined.

Results: For exam-1, the median (lower quartile, upper quartile) of BCS was 3.0 (2.75, 3.25) for observer 3, and 3.25 (3.0, 3.25) for both observers 1 and 2. For exam-2, the median (lower quartile, upper quartile) of BCS was 3.0 (2.75, 3.25) for the three observers. The κ values for BCS measurements were 0.79 (95% CI: 0.69-0.85) and 0.84 (95% CI: 0.77-0.89) for exam-1 and exam-2, respectively. These results suggested a strong agreement between observers on single BCS measurements. The median (lower quartile, upper quartile) of DMBCS between exam-1 and exam-2 was 0.25 (0.0, 0.25) for observer 1, and 0.25 (0.0, 0.50) for both observers 2 and 3. These results suggested a slight loss of BCS over time in cows. When assessing agreement on DMBCS difference, a κ value of 0.49 (95% CI: 0.32-0.63) was obtained suggesting a moderate agreement between observers.

Conclusions: These results showed a lower agreement between observers when assessing DMBCS instead of independent BCS measurements. In this study, the median of DMBCS was 0.25 point and the range was relatively narrow (0.5). Therefore, such a small discrepancy between observers can still produce substantial disagreement. This finding suggests that multiple experienced observers could assess the BCS if only done once in scientific studies or in herds. However, in the same situations but using it to evaluate DMBCS (e.g. monitoring of the negative energy balance), assessment by one single observer would provide more reliable results.

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NU-016-002

Identification of mycotoxin concentrations associated with disease and reproduction in dairy cows

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Objectives: Mycotoxins are biological compounds produced by microscopic fungi called molds. In the province of Québec (Canada), deoxynivalenol (DON) and zearalenon (ZEN) are the most prevalent mycotoxins in dairy cows feed. Acute intoxications with DON or ZEN are reported to have negative impacts on health and reproduction of dairy cows. However, data about the impacts of chronic low-dose intoxications of dairy cows with these mycotoxins are lacking. Therefore, the objective of this study was to identify DON and ZEN concentrations associated with disease or reproduction.

Materials and Methods: A prospective cohort study was conducted on 30 commercial dairy herds located in the vicinity of the bovine ambulatory clinic of the Faculté de médecine vétérinaire of the Université de Montréal (Saint-Hyacinthe, QC, Canada). A total of 360 dairy cows were enrolled in the study (12 cows per herd). Participating herds were conveniently selected based on their willingness to participate and on having computerized health and reproduction records. Herds were visited once by a research technician to collect feed, urine and blood samples (coccygeal vessels) from participating cows. These samples were tested for DON, ZEN, alpha-ZEN and beta-ZEN at the diagnostic laboratory of the Université de Montréal (Saint-Hyacinthe, QC, Canada). Cows were followed for 300 days after calving for disease occurrence and reproductive performance. Statistical analyses were performed using multivariable mixed logistic regression models.

Results: Cows fed ≥ 6.74 and ≥ 3.21 parts per million of DON were 4 and 2 times more likely, respectively, to abort and to have purulent vaginal discharge (clinical endometritis) than cows exposed to lower concentrations ($P < 0.01$). Cows with urinary concentration of beta-ZEN ≥ 6.23 parts per billion were 2 times more likely to be culled than cows exposed to lower concentrations ($P < 0.01$). Cows with urinary concentration of ZEN ≥ 2.70 parts per billion had 0.5 times the odds of conceiving at inseminations compared to cows with lower concentrations ($P < 0.01$).

Conclusions: Overall, these results suggest that feed and urinary concentration thresholds of DON and ZEN can be used to identify cows at high risk of disease and low risk of conception. These results should help farmers, veterinarians, and nutritionists to manage the impact of DON and ZEN in dairy cows.

NU-016-003

Trace element deficiencies related to production or health disorders in Belgian cattle herds

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Objectives: trace elements (TE) deficiencies impair health status of cattle herds and are described to be widely spread in Belgium. TE supplementation during winter is rarely accurately measured. During

pasture, TE supplementation is not systematically performed, especially for beef breeds or pregnant animals and heifers. The objective of this study was to bring out the TE status of dairy and beef herds visited for production, reproduction, mastitis or neonatal troubles, regardless TE supplementation. Furthermore, a comparison of the severity of TE deficiencies between years, seasons, speculations and agricultural regions was made.

Materials and Methods: From February 2014 to October 2015, blood sampling (heparin and plain tubes) were made in 110 cattle herds investigated for health or production disorders in Wallonia. In each farm, 7-10 clinically healthy animals, belonging to a specific and homogeneous group (production, heifers, pregnant), were sampled. Blood was centrifuged to harvest plasma and serum. Hemolyzed plasma/sera were discarded. To rule out the influence of an inflammatory process, the total proteins (TP) in both serum and plasma were measured using an electronic refractometer (EUROMEX®) and the fibrinogen concentration was so estimated. If suspicion of inflammation was present (difference between plasma TP and serum TP >6 g/L), the sample was also rejected. In order to limit costs of analysis, serum/plasma were pooled within a group/farm, so only one serum/plasma sample per group/farm was assayed. Determination of selenium (Se), zinc (Zn), Copper (Cu), iodine (I), vitamins (Vit) (A, E, B12) and haptoglobin concentration in plasma/serum was made in the laboratory (Collard-Synlab). Statistical evaluation, using average, standard deviation, proportion of deficient herds and t-Test, allowed the comparison of groups of production, speculation, year, season and agricultural regions regarding TE status.

Results: Se deficiency was the most represented as only 16% of herds were tested with an adequate Se status. The average Se concentration was 61 ± 20 µg/L (mean \pm SD). In average, the blood Cu, Zn, and vitamin A/E/B12 concentrations were adequate. However, 22, 33 and 52% of the herds showed respectively an inadequate Cu, Vit B12 and Zn status. For Se status, there was a significant ($P < 0.05$) difference regarding the type of speculation with a higher level for the dairy compared to beef herds (Blood Se concentration of 64.0 ± 17.6 vs 55.4 ± 24.3 µg/L and 17 vs 11% of herds with a good status). For Cu, the difference was also significant ($P < 0.05$) in favor of the dairy herds. Samples from cows in production (dairy and beef) had a significantly ($P < 0.05$) higher Se concentration than the non-productive animals (heifers and pregnant cows) (66 vs $51 \pm \dots$ µg/L). In 2014, the global Se status was worse than in 2015 (57 ± 21 vs 66 ± 20 µg/L, $P < 0.05$) while Cu ($P < 0.01$), Zn ($P < 0.05$), I ($P < 0.05$), VitB12 ("trend" $P = 0.06$) and Vit A ($P < 0.05$) blood concentrations were lower in 2015 compared to 2014. Vit B12 deficiency was startling because normally ruminant is not subject to vit B deficiencies, however, in 2015 42% of the herds were deficient regardless of speculation and production. As vitamin B12 is related to rumen health and its bacterial activity, the hypothesis of better quality roughage ingested in 2014 was raised. Finally, a better selenium status ($P < 0.05$) was found during spring compared to autumn, probably due to the better distribution of mineral supplements during the winter period.

Conclusions: Despite massive information to farmers regarding TE importance and previous studies, it appears that Se deficiency remains quite important. Moreover, pregnant cows and heifers are more susceptible to these deficiencies. This results may be explained by insufficient TE recommendations for highly performant cattle such as Holstein or Belgian Blue cows, TE content allowed in mineral formulations, and the difficult economic situation in agriculture. TE status still need to be investigated in herds with production, reproduction or health problems, whatever the mineral supplementation occurring in the farm.

NU-016-004

Muscle function and muscle tissue phosphorus content in transiently phosphorus deprived dairy cows

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Objectives: The clinical relevance of hypophosphatemia that is commonly found in periparturient and anorectic cattle is uncertain but has empirically been associated with persistent recumbency in dairy cows. The objective of the present study was to determine if transient dietary phosphorus (P) deprivation of lactating dairy cows results in altered muscle function or muscle P metabolism.

Materials and Methods: Ten healthy multiparous, mid-lactating dairy cows received a ration with a P content of 0.18% over a period of 5 weeks that was followed by 2-weeks P supplementation phase. Blood, urine and muscle biopsy samples were collected regularly. Function of skeletal and heart muscles were evaluated regularly by electrocardiography and electromyography. Time effects were identified by repeated measures ANOVA.

Results: Dietary P deprivation resulted in the rapid development of marked hypophosphatemia. Lowest plasma [P_i] were measured after 9 days and were on average 60% below baseline. None of the animals developed clinical signs commonly associated with hypophosphatemia or any other health issues. Biochemical muscle tissue analysis showed that dietary P depletion and hypophosphatemia were not associated with a decline in muscle tissue P content. Electromyographic examination revealed increased occurrence of pathological spontaneous activity in striated muscles after 2 weeks of dietary P depletion in several cows, which could be suggestive of neuromuscular membrane instability.

Conclusions: These results suggest that muscle function was not grossly affected, although the increased occurrence of pathological spontaneous activity suggests that subclinical neuro-myopathy may have occurred. The results presented here furthermore indicate that plasma [P_i] is unsuitable to assess the muscle tissue P content in cattle.

NU-016-005

Changes in the Fecal Microbiota of Beef Cattle Caused by Confinement and the Use of Virginiamycin as Growth Promoter.

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Objectives: This study aimed to use high throughput sequencing to characterize longitudinal changes in the fecal microbiota of beef cattle from entrance to exit of a feedlot operation, as well as the consequences of virginiamycin used as Antibiotic Growth Promoter (AGP).

Materials and Methods: Thirty beef cattle entering a feedlot operation were randomly assigned into a treatment group receiving virginiamycin (ATB) for an estimated intake of 0.94 mg/kg/day, or into a control group

receiving the same diet without any antibiotic (CON). Fecal samples were collected at arrival, mid feedlot (25 days after arrival) and at exit to slaughter. The V4 region of the 16S rRNA gene was sequenced with an Illumina MiSeq platform. Bacterial membership (the different species composing the microbiota) and structure (bacterial composition and evenness of each species) were addressed respectively by the Classical Jaccard and the Yue and Clayton indices. A P-test was used to compare membership and structure found at each sampling time, and also for the comparison between groups (ATB vs. CON).

Results: Transition to confinement housing and management caused marked changes in membership and structure of fecal microbiota of beef cattle. At the phylum level, the comparison between arrival and mid feedlot revealed that entrance into the feedlot led to an increase of Firmicutes ($P=0.002$), Proteobacteria ($P=0.010$), Actinobacteria and Spirochetes and a decrease of Bacteroidetes, and Verrucomicrobia ($P<0.001$ for all comparisons). The use of virginiamycin significantly affected only bacterial membership, suggesting that changes mainly involved rare members of the community, and not the most abundant species present in the distal gut of feedlot cattle. At mid feedlot period, only Spirochaetes in ATB were significantly greater than in CON ($P<0.001$).

Conclusions: This study adds new insights to the understanding of microbial dynamics occurring due to changes in diet and the use of AGPs in feedlot cattle. The marked changes in composition and structure of fecal microbiota of feedlot cattle caused by change in management were characterized. The use of virginiamycin as AGP significantly affected membership of the fecal bacteria of beef cattle suggesting that the antibiotic has a stronger effect on the rare species present in that environment, but not on the most abundant species.

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PS1-042-001

Phenotypic and Genetic variation of Liver Fluke Infection in Irish Cattle

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Objectives: Infection with liver fluke is estimated to cost the Irish cattle industry between €70-90 million every year due to losses in milk and meat production and treatment of acute cases. Complications of infection with liver fluke can include: anaemia, secondary infections, cirrhosis, and sudden death. Due to its complicated lifecycle and resistance to environmental exposure, infection with liver fluke can be difficult to control in endemic areas and thus a genetic approach may be a viable alternative. The objective of this paper was to analyse the impact of liver fluke infection on carcass quality and estimate its heritability variance components.

Materials and Methods: The Irish Cattle Breeding Federation (ICBF) engaged two abattoirs with multiple factories around Ireland to pilot an automated data collection program for bovine liver fluke. In this process, veterinary inspectors recorded the incidence of live flukes in a liver, evidence of fluke activity in a liver-but no live flukes, or no fluke activity-past or present. These categories formed the basis of the phenotypic study while the genetic study used infected (past or present) and uninfected as a basis for the heritability estimate. The main edits applied to the dataset for genetic analysis were to include only animals of Holstein-Friesian origin, contemporary groups with some incidence of fluke infection and multiple sires and only animals with sires who had a minimum of 10 progeny distributed across a minimum of 2 herds. The edited dataset and pedigree contained 15,759 and 86,719 animals across 3 categories of animal slaughtered (steers: n = 8,532, young bulls: n = 1,588, cows: n = 5,639) respectively. Carcass information (carcass weight, conformation, and fat grading) was retrieved from the ICBF database for every animals kept. The genetic model applied to the data was a univariate animal model. Phenotypic data was analysed with MS Excel and SAS.

Results: Animals that were infected with fluke often performed worse than animals that were not infected with fluke. Those that were treated for fluke fell into the mid-range of these categories, as we would expect. On average, animals infected with liver fluke took longer to reach slaughter weight, had lower average daily gains, and had poorer carcass conformation than their contemporaries that were never infected with fluke. The heritability of liver fluke infection was 6% for steers, 5% for young bulls, and 2% for cows. Variability of the heritability increased when observing multiple breeds. This result will be investigated with further data on multiple breeds. Genetic correlations between the categories were positive (0.35 to 0.98) but not significant.

Conclusions: The objective of this paper was to analyse the impact of liver fluke infection on carcass quality and estimate its heritability variance components. Liver fluke infection has a negative effect on carcass quality. The heritability presented in this study showed that there is ample genetic variation to start engaging in a genetic program against liver fluke infection. An alternative strategy maybe to breed for resilience in these populations due to a number of infected animals performing as well or better than the uninfected animals, though more studies would have to be done with larger group sizes to ensure adequate power for the study.

PS1-042-002

A Fecal Egg Count Reduction Test With Cattle Treated 120 Days Earlier With Albendazole In Combination With Doramectin Or Extended Release Eprinomectin

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Objectives: At present, cattle in the replacement and stocker phases of production in the USA commonly receive repeated anthelmintic treatments in an attempt by producers to maximize animal performance. As a consequence of this extensive anthelmintic usage, there has been widespread selection for resistance by most nematode parasite species that have a high incidence in younger animals. The current study was conducted to determine the anthelmintic efficacies of certain macrocyclic lactones in stocker cattle that had an immediate history of contrasting chemical intervention and parasite challenge.

Materials and Methods: A fecal egg count reduction test (FECRT) was conducted on naturally infected and heavily challenged Arkansas stocker cattle treated 120 days earlier with saline (S), doramectin in combination with albendazole (DA), or the extended-release formulation of eprinomectin (ER), and grazed by treatment group until the start of the FECRT. Animals within each of the prior groups were blocked by current fecal egg counts, and randomly allocated within each block to treatment with ivermectin, doramectin or moxidectin (all as injectables at 200 mcg/kg BW and with 29-30 animals per treatment group). At 16 days post-treatment, the animals were again fecal sampled for the post-treatment fecal egg count determinations. Additionally, coprocultures were conducted on all individual animal samples collected on days 0 and 16 wherein sufficient nematode eggs were present to allow for successful culturing and harvest. All data were obtained by personnel blinded to animal treatment. It should be noted however, that FECRT efficacies can be drastically divorced from true (control study) efficacies, and hence these data must be considered with that reservation.

Results: On day 0 of the FECRT, the arithmetic mean (SE), strongyle eggs per gram of feces (EPG) counts were 435(108), 561(134) and 341(67), for the ivermectin, doramectin and moxidectin treated cattle, respectively (P>0.94). Day 16 egg counts in the same order were 189(55), 334(98) and 27(15) (P>0.01). Using arithmetic means and by standard equation, the mean FECRT percentages were 56.5, 40.3 and 92.0 for ivermectin, doramectin and moxidectin, respectively. Mean, treatment group-specific FECRT percentages relative to prior experimental group for ivermectin, doramectin and moxidectin were 75, 67 and 95 (S group), 61, 44 and 86 (DA group) and 65, -58 and 92 (ER group), respectively. Coproculture larvae populations harvested on both days 0 and 16 of the FECRT study were *Haemonchus placei* > *Cooperia punctata* > *Oesophagostomum radiatum*, with trace levels of *C. oncophora*, *Trichostrongylus axei* and *Ostertagia ostertagi*. Coproculture, nematode populations were not seen to consistently vary with an animal's anthelmintic history or current parasiticide treatment.

Conclusions: Several observations can be drawn from these data; (1) that anthelmintic efficacies are significantly impacted by prior anthelmintic history, (2) that ivermectin and doramectin efficacies become more depressed than moxidectin efficacy in stocker cattle receiving extensive anthelmintic intervention and nematode challenge, and (3) assuming that 90% fecal egg count reductions are the minimum for an anthelmintic to be considered efficacious, most nematode parasitisms encountered in this study were left unchecked after animal treatment. These study data highlight a pressing concern relative to nematode control and productivity in cattle.

PS1-042-003

Use Of Fenbendazole Medicated Urea Molasses Blocks For Management Of Toxocara Vitulorum In Smallholder Large Ruminant Systems In Developing Countries

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Objectives: *Toxocara vitulorum* (Tv) is a major cause of calf morbidity and mortality in tropical smallholder large ruminant livestock systems, with anecdotal losses of up to 50% of calves in some countries. Although a single treatment of calves with pyrantel between 14 and 21 days following birth has high level efficacy against Tv, this strategy is poorly implemented when external projects cease. An alternative control strategy for Tv is required and the efficacy of treating post-parturient dams with medicated (containing fenbendazole; FBZ) medicated urea molasses blocks (MUMB) was examined.

Materials and Methods: Studies continue in the northern Lao Peoples Democratic Republic (Lao PDR) in 2015-16, following an initial pilot study involving cows (n=66) and calves aged <4 months (n=17), following a pre-treatment Tv prevalence in calves of 29.4% (established by faecal sample examination using a modified McMaster egg counting technique to examine for Tv and strongyle eggs). The effect of MUMB and non-medicated urea molasses blocks (UMB) on the cattle was evaluated through follow-up faecal collections on days 7, 18, and 28 of block supplementation of animals aged between 1 month and 10 years, with faecal egg count, body condition score (BCS), girth, coat texture and estimated price of large ruminants (AUD) recorded for individual animals at time of sampling.

Results: Large ruminant unweaned calves aged <4 months with access to MUMB demonstrated a reduction in mean Tv egg count from 190 eggs per gram (EPG) of faeces at pre-treatment to 0 EPG at day 7 of treatment. At conclusion of the pilot trial on day 28, MUMB and UMB supplementation demonstrated 100% and 95% reduction in large ruminant *T. vitulorum* egg burdens, respectively. From 7 days of block introduction, large ruminants with access to MUMB had a significantly lower (p<0.05) strongyle egg count (13.2 EPG) compared to animals exposed to UMB treatment (41 EPG), with animal accessing UMB supplementation displaying a continuous reduction in strongyle egg burden throughout the trial period. The mean large ruminant weight within both treatment groups increased throughout the trial, with a greater (p<0.001) weight associated with animals exposed to MUMB treatment, although BCS, coat texture and price demonstrated minimal or no change within this pilot study.

Conclusions: This pilot study demonstrates the potential efficacy of MUMB supplementation for control of Tv infection in large ruminant herds and may be more appropriate approach in developing countries than individual calf treatment with pyrantel. Studies are continuing and additional data on evaluation of the potential role of MUMB and UMB in Tv and strongyle parasite suppression will be presented.

Comments: This innovation may offer a more sustainable strategic parasite management strategy for countries where farmer knowledge of parasites is very poor and not easily addressed plus facilities for animal restraint for administration of therapeutics are largely non-existent.

PS1-042-004

Evaluation of a protocol of Paromomycin oral administration regarding the control of neonatal cryptosporidiosis in ruminants

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Objectives: The aim of the trials is to evaluate the efficacy in field conditions of a proposed protocol of Paromomycin oral administration regarding the control of neonatal cryptosporidiosis in calves, lambs and goat kids.

Cryptosporidiosis is a poorly controlled disease: no vaccine is available and there is only one molecule licensed for calves to date (Halocur™) with usage constraints. Published literature supports that Paromomycin has anti-protozoal properties and shows efficacy in the control of cryptosporidiosis in ruminants. Many studies nevertheless have been conducted under experimental infection and wide ranging doses have been recommended.

Materials and Methods: Trials are conducted in France in 3 different farms: a cattle operation, a sheep farm and a caprine holding during winter 2015.

The identified farms have had a history of neonatal scours caused by *Cryptosporidium* spp. with important losses. The attending veterinarian confirmed the diagnosis through faecal analysis by rapid tests in farm based on immunochromatography (Huve-Check®). In each operation, the diarrhoea occurrence determines date starting of the trial (Day 0)

24 animals born on date starting onwards are included in the study in each farm. The maximal interval between birth times of the animals is 48h for lambs and kids, one week for the calves.

They are divided into two groups (treated group and untreated): only males are included in the study, randomly and alternatively assigned to one group or another.

In each farm, the animals receive a dose of powder (Paromomycin sulfate in the treated group or Placebo in the untreated one) via the milk/milk replacer at 50mg/kg once a day (morning or evening, based on the farm regime) for 5 days, starting at 5th day after birth.

All animals enrolled in the experiment are monitored for a period of 28 days and the observations recorded : weight, faecal score 0-1 (absence or presence of diarrhoea), health score 0-1-2 (good, bad, very bad). Every week, starting from the first day of treatment, three faeces samples are taken to assess the presence of *Cryptosporidium* oocysts using flotation method, with an intensity scale (+, ++, +++)

Data analysis will be performed using IBM SPSS Statistics 21

Results: Expected Results

The selected criteria for assessing the efficacy of the protocol of treatment are the prevalence and the intensity of oocyst excretion, the presence of diarrhoea, the health rate status and weight gain.

The comparison of the results of faecal samples should show a different pattern of oocysts excretion between treated and untreated animals. In order to determine possibly differences depending on species, the oocysts excretion curves will also be compared for calves, lambs, goat kids.

For each species, the effectiveness of the treatment will be assess by comparison with the control group of the rate of morbidity and/or mortality, the occurrence and duration of diarrhoea episodes and the evolution according to the age of the animals, the prevalence of clinical signs and level of health score, the improved daily weight gain.

Conclusions: Due to the high resistance of *Cryptosporidium* against a lot of disinfectants and environmental influences, additional appropriate measures should be taken to reduce the prevalence of cryptosporidiosis

If determined to be effective in the study, the protocol of oral administration of a daily dose of 50mg Paromomycin sulfate /kg body weight from the 5th day to the 10th day after birth should allow to control cryptosporidiosis.

This reasonable use of Paromomycin could be recommended in farms experiencing cryptosporidiosis in order to reduce economic losses, environmental contamination and the risks for animal and human health.

Comments: On-going field trials during winter 2015. Reason for which results are "expected" and figures not yet shown.

PS1-042-005

Best practice liver fluke control using synergistic flukicides

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Objectives: Treatment of liver flukes (*Fasciola hepatica*) has been relatively effective since triclobandazole became available in the 1980s, but Boray demonstrated that combinations of actives could provide even better efficacy through truly synergistic interactions. Two such combinations, triclobandazole + oxfendazole (Flukazole® C) and nitroxynil + clorsulon [+ivermectin] (Nitromec® Injection) are available commercially in Australia. The objectives of this work were to determine if the superior efficacy displayed by these synergistic flukicides provides real benefits to livestock producers through better productivity and improved resistance management.

Materials and Methods: Productivity Trials – Young beef cattle (about 9 months old) naturally infected with liver flukes were randomly allocated to groups (n=20-22 per group), individually weighed and then treated according to label instructions with one of the synergistic products or the producer's normal fluke treatment. Case study 1 included triclobandazole (Fasinex®) and triclobandazole + oxfendazole (Flukazole® C) while Case study 2 included triclobandazole + abamectin (Genesis® Ultra Pour-on), triclobandazole + oxfendazole (Flukazole® C) and nitroxynil + clorsulon [+ivermectin] (Nitromec® Injection). All groups received roundworm treatment via the macrocyclic lactone (ML) component of the product or an additional ML treatment (Cydectin® pour-on). An additional triclobandazole + oxfendazole group was treated with a long-acting ML product (Cydectin® LA Injection) to compare the relative impacts of fluke and roundworm control. Fluke egg counts and liveweight gains were then monitored for about 4 months (3 or 4 time points), with the animals being managed as they would normally.

Resistance assessment (Case study 3) – Animals proven to be infected with liver flukes using faecal egg counts were allocated randomly to groups of 15, individually weighed and then either treated according to label instructions with one of three products, triclobandazole (Fasinex®), triclobandazole + oxfendazole (Flukazole® C) or nitroxynil + clorsulon [+ivermectin] Nitromec® Injection, or left untreated (control). Fluke egg counts were performed on Days 0 and 28 as an indicator of reduction in fluke burdens.

Results: Case study 1 – Fluke egg counts were reduced to zero in all animals and remained that way throughout the trial period. Despite that, animals treated with the triclobandazole + oxfendazole combination gained weight faster than those receiving triclobandazole alone. At 135 days after treatment an average weight advantage of 8kg (liveweight gain of 52kg vs 44kg) was observed (P<0.05). In comparison, an extra 13kg in liveweight gain was achieved by including better roundworm control.

Case Study 2 – Fluke eggs were detected in the faeces of animals in the pour-on group at Day 71 whereas eggs were detected in the triclobandazole + oxfendazole group at Day 139 and the injection group remained at zero throughout. In this case the synergised flukicides provided a weight gain advantage of 11-12 kg (54kg and 55kg vs 43kg) (P<0.10) while better roundworm control provided a benefit of 7kg.

Case study 3 – Fluke egg counts remained high in the control group (11.9 eggs/10g at Day 28). While the number of animals positive by egg count in the group treated with triclobandazole alone was lower than that in the control group, the mean egg count in the positive animals was higher than the control group (13.1 eggs/10g). While the triclobandazole + oxfendazole treatment was not fully effective, that group had even fewer animals testing positive and the mean egg count in positive animals was low (3.5 eggs/10g). In contrast, the nitroxynil + clorsulon treatment was highly effective (only 1 egg detected in one animal).

Conclusions: Synergistic flukicides can provide substantial productivity benefits to livestock producers and could also play an important role in managing triclobandazole resistance. While no triclobandazole-based product could be recommended for use against resistant flukes, the principle of using the highest potency products to minimise selection for resistance via 'head selection' suggests that combinations should be integral in preventative programs. The nitroxynil + clorsulon combination offers a highly efficacious alternative for use in rotational programs to slow the onset of resistance or to overcome flukes already resistant to triclobandazole.

PS1-042-006

Acquired immunity towards digestive strongyles in 2nd grazing season dairy heifers not dewormed with macrocyclic lactones during the previous winter

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Objectives: The aim of the study is to demonstrate that the absence of deworming at housing with an antiparasitic macrolide allows, during the winter period, the accomplishment of immunity towards digestive strongyles (GIS) in dairy heifers during their second grazing season. Such a strategy allows heifers to meet a complete immunity towards fourth stages and adults worms of GIS during the winter. So, it will not be necessary to deworm them during the second grazing season. The next antiparasitic treatment will usually take place at calving.

Materials and Methods: Six herds (1 in 2014, 5 in 2015) were selected because they were followed up according to parasitology audit design in our practice; that consists in a quality approach aiming to assess and manage as precisely as possible the parasitic risk.

Data regarding the first grazing season were available (pasture management, anthelmintic treatments). Those herds were implementing preventive treatments (long acting or pour-on moxidectin, oxfendazole boluses).

In those herds, blood pepsinogen have been measured at the end of the first grazing season according to the French method, allowing us to determine the best choice of anthelmintic housing treatment. None was advised for 4 herds and oral fenbendazole for 2.

During the second grazing season, fecal egg counts were performed 10 to 16 weeks after turn out using a Mc Master method with a sensitivity of approximately 10 epg (egg per gram of faeces) ; 5 heifers were investigated within 5 herds, 3 within the last one.

Results: Among the 28 investigated heifers, 21 were not shedding any eggs, the remaining seven were shedding between 10 and 20 epg. The detailed results for each herd are the followings:

Houdetot (2014) : 1st grazing season (GS), 2 batches turned out mid-April & mid-May (long-acting [LA] moxidectin (mox.) at beginning of April. Housed mid-November; 7 Pepsinogen **Results:** 420 > 821 ; no treatment. 2nd GS, turn out at beginning of April, full grass, FEC: 0, 0, 0, 10, 18

Bréant (2015) : 1st GS, 2 batches turned out mid-april (oxfendazole bolus) & mid-august (LA mox.). Housed beginning of November; no Pepsinogen results ; no treatment. 2nd GS, turn out beginning of May, two plots with fortnight movement, FEC: 5 negatives

Eudier (2015) : 1st GS, 2 batches turned out mid-April (LA mox.) & mid-June (pour-on mox.). Housed at the end of November; 5 Pepsinogen: average 1063 (475 > 2085); Fenbendazole per os. 2nd GS, turn out beginning of April, full grass, FEC: 0, 0, 0, 0, 18

B. Leber (2015) : 1st GS, turn out on April or May, LA mox. at the end of March. Housed 20th November; 5 Pepsinogen: average 1170 (586 > 1852); no treatment. 2nd GS, turn out 20th April, full grass, FEC: 3 negatives

Chêne (2015) : 1st GS, LA or pour-on mox. according to the time of turnout. Housed end of November; 7 Pepsinogen: average 1130 (656 > 1376) ; Fenbendazole per os. 2nd GS, turn out at the beginning of April, full grass, FEC: 0, 0, 0, 13, 17

Houdetot (2015) : 1st GS, same management as 2014. 4 Pepsinogen: 597 > 646 ; no treatment. 2nd GS, same management as 2014; FEC 2nd GS: 0, 0, 0, 10, 20

Conclusions: The absence of GIS egg shedding during the second grazing season in not winter dewormed heifers is a good indicator of the acquired immunity toward those parasites. It gives a good assessment of the parasitic challenge met by the animals.

The most important conclusion consists in the fact that a sustainable management of the 1st grazing season GIS infestation allows to drastically reduce the use of anthelmintic treatments during the 2nd grazing season with compromising an optimal growth and first calving size.

PS2-043-001

Can we control nematodes more efficiently in suckler beef herds using targeted selective treatments?

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Objectives: With the emergence of anthelmintic resistance (AR) in cattle nematode populations, there is a need to reassess our currently employed approaches to nematode control in cattle. One potential solution is to focus on treatment of the individual animal using targeted selective treatments (TSTs). This study reports on the use of a TST-based approach to nematode control in suckler beef calves over their first grazing season (FGS). The objective was to determine the minimum number of anthelmintic treatments needed to control infections due to both *Dictyocaulus viviparus* (lungworm) and *Ostertagia ostertagi* in FGS suckler beef calves.

Materials and Methods: Ninety nine beef calves with an initial mean age (s.d.) and mean live weight (s.d.) on June 28th (day 0) of 107 (23.1) days and 160 (32.5) kg, respectively, from a spring-calving beef herd were used in this study. Calves with their dams were randomised to one of two treatments; 1), standard treatment (positive control) (n = 25; × 2) and 2), TST (n = 25; × 1 and n = 24; × 1) based on calf age, weight, sex, dam breed and sire breed. Control and TST calves and their dams were rotationally grazed in adjacent paddocks. The study commenced on day 0 and finished on day 124. Every three weeks calves were weighed and blood and faecal sampled. Faecal samples were analysed for the presence of lungworm larvae using a modified Baermann technique and faecal egg counts (FEC) were determined using a modified McMaster method. Plasma pepsinogen concentrations (PP) were also measured while blood samples collected from calves on days 0 and 124 were tested for the presence of antibodies to lungworm using an enzyme-linked immunosorbent assay (ELISA). All calves in the control groups were treated subcutaneously with ivermectin on days 0, 41 and 82 while all calves (both control and TST) were treated with ivermectin on day 124. Individual calves in the TST groups were treated with ivermectin based on their laboratory results as follows; 1), a positive Baermann result or 2), a positive/negative Baermann result with PP ≥ 2 international units of tyrosine per litre and FEC ≥ 200 eggs per gram of faeces. Data were analysed using the PROC MIXED function of SAS (Version 9.3, SAS Institute, Cary, NC).

Results: Apart from the pre-housing treatment, no calves in the TST groups were treated at pasture. The overall average daily live weight gain (P = 0.17) for control and TST groups was 0.89 (± 0.02 SEM) kg/day and 0.94 (± 0.02 SEM) kg/day, respectively. There was an effect of time (P = 0.005), treatment (P < 0.001) and a treatment × time interaction (P = 0.004) for FEC. There was an effect of time (P < 0.001), a treatment × time interaction (P < 0.001) and no effect of treatment (P = 0.535) for PP. With an optical density ratio ≥ 0.5 indicating infection with adult lungworm, three calves (two control and one TST) were seropositive on day 0. This was in contrast to day 124 when 28 calves (18 TST and 10 control) were seropositive despite no calves having lungworm larvae identified in their faeces during the course of the study.

Conclusions: Nematode challenge can potentially be controlled with minimal anthelmintic treatments in FGS suckler beef calves at pasture whilst not significantly impairing performance. In addition, although suckler beef calves at pasture do get exposed to lungworm challenge in the FGS, this appears to occur at low levels as judged by the differences in results between the ELISA and the modified Baermann technique.

PS2-043-002

Detection of acute *Tritrichomonas foetus* infection in beef cows and bulls

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Objectives: *Trichostrongylus axei* causes a highly contagious venereal disease of cattle worldwide whose control relies on detection of infected bulls. The purpose of this study was to examine the detection of early infections in cows and bulls.

Materials and Methods: Eight *T. axei* negative cows were bred by an infected bull. Post exposure vaginal samples were collected daily for one week, then twice in a week, and then once weekly for five weeks. Eight *T. axei* test negative bulls bred an infected cow and preputial samples were collected on days 1, 2, 3, 4, 5, 6, 7, 14, 19, 22, and 25 post exposure.

Samples were incubated for 4 days with daily microscopic examination followed by polymerase chain reaction testing.

A spreadsheet calculated the descriptive statistics.

Results: Eight cows became infected with median and mean days to initial diagnosis of 4 and 7.8 days, respectively and median and mean days to continuous positive tests of 10.5 and 11.9 days, respectively. Days to initial detection in cows ranged from 1 and 28 days.

Three bulls became infected with median and mean days to initial diagnosis of 4, 3.7 days, respectively and median and mean days to continuous positive tests of 19 and 17.9 days, respectively. Days to initial detection in bulls ranged from 1 and 4 days.

Conclusions: Testing acutely infected cows may provide early detection of herd infections. Acutely infected bulls generate positive tests, but may require more than 2 weeks of sexual rest for consistent test positivity.

Comments: This abstract is less than 250 words to protect future publication options for the full manuscript. Unfortunately this makes it extremely brief.

PS2-043-003

The development and application of a protocol to identify biases in papers on abattoir surveys of *Fasciola hepatica* infection in ruminants

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Objectives: *Fasciola hepatica* is a parasite of clinical and economic importance in ruminants worldwide and in parts of the world is also an important zoonotic pathogen. Abattoir surveys are often used to estimate the prevalence of *F. hepatica* in ruminants because they allow researchers to inexpensively study large samples. Nevertheless, prevalence estimates from these surveys can be affected by both selection and information biases. The aim of this project was to develop and apply a protocol, to recently published papers on abattoir surveys of *F. hepatica*, in order to determine the extent to which these biases were taken into consideration.

Materials and Methods: We first developed a series of questions aimed at determining if selection and/or information bias are present in published abattoir surveys. The questions were developed as a flow chart and aimed to analyse whether the sample in the abattoir reflected the population to which inferences are being made, whether the movement history of the animals is discussed and whether the paper adequately outlines the procedure used to determine *F. hepatica* infection. Papers

for analysis were selected by conducting a literature review in PubMed. Search terms used were "liver fluke and abattoir and prevalence" with the terms present in either the title or the abstract. We included papers published between January 2005 and January 2015 in our study, giving 28 papers for review. Papers were further excluded for the following reasons: the main focus was on other parasite species (7), prevalence was not discussed (4), the paper was not available in English (4), or at all (1). We then applied the questions, in the form of a flow chart, to the remaining 12 papers to check for the presence of selection and/or information bias.

Results: The flow chart protocol made the procedure for applying questions to papers a streamlined and systematic process. The questions posed identified that the population to which inferences were made was thoroughly discussed in 2 papers, there was limited discussion in 9 papers and it was not discussed in 1 paper. The geographic location of the abattoirs was described in all papers and the location from which the animal population was derived was discussed to some extent in 4 papers. The implication in other papers (8) was that the abattoir population of cattle was derived from the region surrounding the abattoir but this was not stated explicitly. The movement history of the animals was not discussed in any of the papers reviewed. The procedure used to determine the infection status of livers was thoroughly described in 7 of the 12 papers reviewed while one paper did not describe the procedure at all. Eight papers did not report the sensitivity or specificity of the test. Two papers briefly discussed the sensitivity of meat inspection for diagnosing *F. hepatica* but did not quantify it and 2 papers considered the techniques they used to be a gold standard for diagnosing *F. hepatica*.

Conclusions: The questions posed in our study identified that most papers did not adequately consider the potential for selection or information bias. Given that the validity of prevalence estimates are determined by the magnitude of these biases, it is prudent to consider their presence in an abattoir survey. While a final verdict on their extent may be a matter of judgement, they still need to be taken into consideration. A systematic method, such as a flow chart-as used here, would allow researchers to identify potential biases in abattoir data. Readers should also consider if papers resulting from such data adequately deal with the subject of bias.

PS2-043-004

Alternative Interpretations of English Faecal Egg Count Reduction Test (FECRT) Data

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Objectives: Anthelmintic (field) efficacy in cattle is most commonly assessed using the (unvalidated) Faecal Egg Count Reduction Test (FECRT). FECRT outcome is influenced by group size, pre-treatment faecal egg count (FEC) and FEC sensitivity. Current WAAVP guidelines don't take full account of these or other factors (such as incorrect dosing or sample handling) risking falsely identifying Efficacy or Lack of Efficacy (LOE, which the current guidelines classify as anthelmintic resistance). Here, two expert systems were designed to aid interpretation of FECRT data sets to reduce the likelihood of misclassifying FECRT results as "LOE" or "Resistance".

Materials and Methods: FEC data from 88 FECRTs conducted in

first-season animals in geographically distinct English regions between 2012 and 2014 were analysed by calculating percentage reductions in egg counts using pre- and post-treatment group arithmetic mean FEC, and 95% confidence intervals (CI) were constructed. The efficacy of macrocyclic lactones (ivermectin and doramectin pour-on & injectable) and fenbendazole (drench) were assessed. The classification of FECRT results using adapted WAAVP thresholds (i.e. efficacy $\geq 95\%$ and lower 95% CI $\geq 90\%$ indicating acceptable anthelmintic efficacy), were compared to the evaluation of the same FECRT data using two unvalidated expert systems based on published literature and personal experience. Expert system 1 (ES1) considers treatment group size, the group mean Day 0 and Day 14 FEC, and the distribution of egg counts. Expert system 2 (ES2), in addition to the criteria applied using ES1, considers the width of the 95% confidence intervals, the larval culture data and the season in which the FECRT was conducted.

Results: When applying WAAVP criteria, 28.4% of groups were classified as efficacious and 71.6% were classified as lack of efficacy (LOE). When ES1 was applied, the percentage of FECRT classified as efficacious and LOE decreased to 8 and 9.1% respectively and 83% were classified as inconclusive. When ES2 was applied, 20.5% of the FECRT results were classified as efficacious, 20.5% as LOE, 25% as suspected (S)LOE and 34.1% as inconclusive. Using WAAVP criteria, 25 groups were classified as efficacious and of these, ES1 categorised 7 as efficacious and 18 as inconclusive, while ES2 categorised 16 as efficacious, 2 as (S)LOE and 7 as Inconclusive (INC). WAAVP criteria classified 63 groups as LOE. Using ES1, 8 were LOE and 55 were inconclusive. Using ES2, 2 groups were efficacious, 19 SLOE, 19 LOE and 30 INC.

Conclusions: Many WAAVP "LOE/Resistance" results were inconclusive when expert systems were applied. ES1 produced more inconclusive results than ES2, suggesting the extra criteria included in ES2 aid the interpretation of FECRT results, reducing the number of inconclusive results. Misclassification of product efficacy may occur if estimates do not take into account eg treatment group size, pre-treatment mean FEC, etc. These unvalidated "Expert Systems" provided a more "granular" assessment of "LOE", allowing apparent LOE to be differentiated from farms with less conclusive evidence, something "standard" WAAVP interpretation did not allow.

PS2-043-005

Six-year longitudinal study of *Fasciola hepatica* bulk milk ELISA testing in Irish dairy herds

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Objectives: Risk factors associated with *Fasciola hepatica* (liver fluke), one of the most important helminth parasites of ruminants, include high levels of rainfall and temperatures in excess of 10°C. Ireland's weather, therefore, provides ideal climatic conditions under which liver fluke can thrive. Previous studies have highlighted that seasonal variations in liver fluke herd prevalence occur in Ireland. The objective of the current study was to investigate the extent of any annual variation in *F. hepatica* bulk milk ELISA results and examine the correlation between liver fluke bulk milk seropositivity and soil temperature and rainfall.

Materials and Methods: A total of 32 herds participated in the study from March 2009 to December 2014. Of these 22 were commercial farms located in the Munster province of Ireland (southern-western

region). The remaining 10 herds were research farms managed by Teagasc (Irish Agriculture and Food Development Authority). Bulk tank milk (BTM) samples were submitted by each farm on a monthly basis. Samples were collected into standardised kits provided to each farmer. All BTM samples were preserved using broad spectrum microtabs II and received for testing within 48 hours of sampling. A commercially available ELISA kit (Ildana Biotech, Dublin, Ireland) was used to test all samples and has a sensitivity [Se] and specificity [Sp] of 98%. This kit uses a recombinant mutant Cathepsin L1 antigen (CL1), the immunodominant protease found in *F. hepatica*. Each test was completed according to kit manufacturer's instructions and a positive cut-off of 15 S/P was used for herd classification as positive (Pos) or negative (Neg). BTM data from October of each year were used for the current analysis. Rainfall and soil temperature data were obtained from the weather measurement station located in Teagasc, Moorepark, Co Cork. Scatter plots (mean S/P vs. rainfall and mean S/P vs. temperature) and R2 values were generated in MS Excel (2010).

Results: A total of 1420 samples from 32 herds was analysed in total. The mean number of samples received per farm per year was seven. The number of herds submitting October samples in each year of the study, the proportion of herds recording positive BTMs and the mean rainfall and temperature for October in each year are outlined in the following table.

	n	% Pos	Mean S/P	Rainfall	Temp
Year 1	29	65.5	105.9	126.7	12.8
Year 2	28	75.0	107.1	82.6	11.0
Year 3	28	67.9	73.4	83.3	12.1
Year 4	27	77.8	64.3	99.3	10.1
Year 5	26	65.4	56.9	150.4	8.6
Year 6	25	60.0	43.9	153.6	12.4

A poor correlation existed between BTM ELISA and rainfall (R²=0.3) and soil temperature (R²=0.09). It should also be noted that the relationship between rainfall and BTM results was unexpected, as ELISA readings decreased with increasing rainfall.

Conclusions: Although the proportion of herds classified as positive remained relatively static over the course of the study, BTM ELISA readings decreased steadily. The lack of an acceptable correlation between rainfall, soil temperature and liver fluke BTM ELISA would suggest that the decreasing BTM fluke results are due to improved liver fluke control on Irish dairy farms.

PT1-039-001

The practical implementation of a risk based Johne's Disease management programme on large dairy herds

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Objectives: This paper will describe the implementation of a Johne's Disease management programme on three different large dairy herds that opted to use a risk management strategy to control endemic Johne's disease. The study considers the reasons for the selections of the use of quarterly milk testing and the management of high risk cows to prevent new infections, and the specific husbandry practices that were implemented to prevent disease spread. Issues with compliance with the Johne's Disease management plan will be discussed, and specific components of the plan that were particularly difficult to implement will be highlighted.

Materials and Methods: Three large dairy herds (650 cows, 320 cow and 180 cows) were engaged with Johne's Disease surveillance and management as part of a regional paratuberculosis awareness campaign. Each farm had a risk assessment to quantify the risks of Johne's Disease entering the herd and spreading within the herd using a web based herd health management tool (myhealthyherd.com.)

Once biosecurity and biocontainment risks had been quantified, the current prevalence of Johne's disease within each herd was estimated by a targeted 30 cow screen, and a future predicted prevalence estimated to help with the choice of control strategy. Each farm opted for a risk based management strategy using quarterly milk antibody elisa testing to identify high risk cows that were likely to be shedding *Mycobacterium avium* paratuberculosis (MAP), and infectious cows were managed to prevent the spread of infection to susceptible young stock

Results: A specific risk management strategy was formulated for each farm to minimise the risk of MAP transferring from infected, infectious cows to susceptible young stock. The plan was created using the known risks identified in the risk assessment, and the practicality of implementing effective husbandry changes that would prevent new infections. The robustness of the plan was tested against a model within the health management system to ensure that there was a high likelihood of success.

Each of the farms found the implementation of the plan challenging. The control of spread was particularly challenging for the herds with large numbers of cows calving in limited calving yards or calving accommodation during the winter months. There were also issues with compliance with milk and colostrum separation and pasteurisation, with several instances of post pasteurisation contamination leading to potential spread of disease. The level of compliance reflected the success of Johne's Disease control, with poor compliance being revealed by first lactation heifers testing positive by milk elisa tests some three years after the implementation of the plan. In one herd the number of test positive first lactation heifers fell from 7.7% to 2%, and in another, it rose from 3.4% to 4.3% over 3-4 years. All herds managed to significantly reduce the prevalence of Johne's Disease compared to the prediction of prevalence at the outset, but none have managed to eradicate the disease completely.

Conclusions: Each of the three farms had significant prevalence of Johne's Disease present in the dairy herds at the start of the programme. Predicted prevalence in all three herds was high due to the high risks of spread inherent with their husbandry practices which was revealed in the risk assessment conducted at the outset. The study demonstrates that risk based control strategies are effective at controlling Johne's Disease in large dairy herds, but the effectiveness of the control measures is

dependent upon compliance with the plan, and the larger herds have the biggest challenges for control.

PT1-039-002

Assessment of the effect of *Mycobacterium avium* subs. paratuberculosis in 305ME production and in udder health using HGLM, in Portuguese dairy cows.

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Objectives: To assess the impact on milk production and udder health of *Mycobacterium avium* subs. Paratuberculosis (MAP) infection in dairy cows in the Portuguese Dairy Control Program (PDCP), taking into account the intensity of infection at herd level.

Materials and Methods: All cows aged over 30 month's age, from 329 farms (in total 15,300 animals) were MAP tested by indirect biphasic ELISA. These cows represent 6.45% of the Portuguese dairy cow's population and 18.5% of cows (and 20% of farms) registered in PDCP. Cows were classified as positive (POS), negative (NEG), and dubious (DUB), based on (23,919 tests/20,221 cows) ELISA results. The farm intensity of infection was defined based on all individual ELISA results available to the farm in four levels: Strongly Negative (SNEG), Negative (NEG), Non-Negative (NNEG) and Positive (POS). Classification of each cow MAP status was performed taking into account its individual result within the farm intensity level in eight categories: NEG_SNEG, NEG cow in SNEG farm; NEG_NEG, negative cow in NEG farm; NEG_NNEG, NEG cow in NNEG farm; NEG_POS, NEG cow in POS farm; DUB_NNEG, DUB cow in NNEG farm; DUB_POS, DUB cow in POS farm; POS_NNEG, POS cow in NNEG farm; POS_POS, POS cow in POS herd. Records from 395,802 somatic cell counts (SCC) and Milk Productions (MP) of 40,065 lactations from 15,300 cows were available. Milk productions 305 day's equivalent (305MEP), average SCC and capability index for SCC (CpKccs) were calculated. Outcome variables: 305MEP and udder health were investigated using PTB status and PTB Level as explanatory variables, calving season as covariate, and farm and lactation number as hierarchical levels in Hierarchical Generalized Lineal Model (HGLM). Data was analyzed with IBM SPSS Statistics, version 23 and SuperMixe 2.1.

Results: Of the total 15,196 cows, 90.9% were NEG, 0.58% were DUB and 4.5% were POS. Correspondingly 4.67% of lactations were also positive. As to farms, 14.6% were S_NEG, 39.0% NEG, 18.2% N_NEG and 29.2% were POS. Positive and non-negative farms totalized 47.4% of observations. Only 17.2% of cows belonged to potentially MAP free farms; 58.3% of the cows were NEG animals in positive farms. The validity of 40,065 lactation records was assessed for NEG, DUB and POS cows, using qui-square test and no difference was found ($p=0.2$). Differences were found between the proportion of valid lactations by farm MAP status, by animal PTB level, and by farm PTB intensity level ($p<0.00$). Regardless of cow status, 62.2% of lactations and calving's, occurred in MAP-POS farms, suggesting high risk of contact with the agent. Only 18.1% of lactations/calvings occurred in MAP free farms. Overall, 305MEP lactation averages were higher in POS farms, than in SNEG, NEG and NNEG farms. Average 305MEP by cow status was higher in NEG than in POS cows for first, third and fourth lactations,

but higher in POS than in NEG for second, fifth, sixth, seventh and eighth lactations. As to PTB level, NEG_POS 305MEP were higher than POS_POS for second, third, fourth and fifth lactations Overall SCC were higher in POS farms than in the remainder, from first to third, sixth and seventh lactations; higher than SNEG and NNEG but lower than NEG for fourth and fifth lactations. Better understanding of these variations was achieved with HGLM.

Conclusions: This study indicates the existence of high prevalence of MAP infection in cows and farms and the potentially intense exposure of cows and calves to MAP infection since more than 60% of calvings and lactations occurred in infected farms. Bulk milk should also be exposed to contamination. The influence of MAP infection in udder health and milk production in Portuguese dairies is assessed.

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PT1-039-003

Dysbiosis of the Fecal Microbiota in Cattle Infected with *Mycobacterium avium* subsp. *paratuberculosis*

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Objectives: The purpose of this study was to investigate the diversity patterns of fecal bacterial populations in cattle infected with *Mycobacterium avium* subsp. *paratuberculosis* (MAP), compared to those of uninfected control cattle, using phylogenomic analysis.

Materials and Methods: Fecal samples were selected from the New Bolton Center Johne's disease Laboratory's fecal repository to include samples from 20 naturally infected, non-clinical MAP-positive cows (positive group); 25 MAP-negative herd mates (exposed group); and 25 MAP-negative cows from a MAP-free herd (negative group).

Fecal samples were processed for genomic DNA extraction, using a commercially available DNA extraction kit. The extracted and purified DNA was amplified for the V1-V2 hyper-variable regions of the 16S rDNA gene using barcoded 8F and 357R primers. The amplicons were then bead purified using 1:1 Agentcourt AmPure XP beads. The purified products from the fecal samples were pooled in equal concentration prior to pyrosequencing. The 16S rDNA sequences obtained were decoded and analyzed using the QIIME pipeline.

Statistical analysis and graphical presentation of data was performed using R program. A non-parametric permutational multivariate ANOVA test, implemented in the vegan package for R, was conducted for each pair of bacterial communities, as measured by weighted UniFrac distance. To test for differences in taxon abundance, a generalized linear mixed model was constructed with the lme4 package for R. The model used a binomial link function and included a random effect term for each sample. The input data for the mixed model consisted of a two-column matrix containing (in column 1) the number of reads assigned to the taxon, and (in column 2) the number of reads assigned to other taxa.

Results: Approximately 2,52,380 reads were analyzed from 70 bacterial communities (average of 2,843 reads/sample). Clustering at 97% sequence similarity produced approximately 34,606 OTUs. Weighted and unweighted UniFrac distances by principal coordinate analysis showed a substantial effect ($P < 0.001$; Permanova test) of groups on the bacterial community composition. Taxonomic assignment of the OTUs identified 18 bacterial phyla across all samples. Bacteroidetes and Firmicutes constituted more than 95% of the bacterial population in the negative

and exposed groups. In the positive group, lineages of Actinobacteria and Proteobacteria increased and those of Bacteroidetes and Firmicutes decreased ($P < 0.001$). Actinobacteria was highly abundant (30% of the total bacteria) in the positive group compared to exposed and negative groups (0.1 – 0.2 %).

Conclusions: Fecal bacterial communities of MAP-positive cows varied significantly from those of cows from the exposed and negative groups. Bacterial communities within the exposed and negative groups were mostly homologous, whereas there was statistically significant greater variation between fecal samples in the MAP-positive cows. These findings are similar to the gastrointestinal dysbiosis demonstrated in patients suffering from various types of inflammatory bowel disease, including Crohn's disease (CD).

Comments: Background: Johne's disease (JD) is a chronic, intestinal infection of cattle, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). It results in granulomatous inflammation of the intestinal lining, leading to malabsorption, diarrhea, and weight loss. Crohn's disease (CD), a chronic, inflammatory gastrointestinal disease of humans, has many clinical and pathologic similarities to JD. Dysbiosis of the enteric microbiota has been demonstrated in CD patients. It is speculated that this dysbiosis may contribute to the intestinal inflammation observed in those patients.

PT1-039-004

The use of *Mycobacterium smegmatis* as a DNA extraction and RT-PCR internal control for the detection of MAP from a bovine faecal environment

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Objectives: *Mycobacterium avium* subspecies *paratuberculosis* (MAP) inflicts large economic losses to the dairy industry worldwide. MAP DNA extraction from a bovine faecal environment has proven to be difficult, due to the resistant nature of the *Mycobacterium* cell wall. The aim of this study was to develop a novel specific MAP DNA extraction and realtime-(RT)-PCR internal control from a faecal environment. The use of *Mycobacterium smegmatis* mc2 155 strain with a molecular internal control (IC) facilitates the confirmation of MAP DNA and the IC by RT-PCR and therefore eliminates false negative results due to inefficient MAP cell lysis.

Materials and Methods: An 844 bp internal section of the ampicillin gene from the pUC19 vector was used for the development of the IC. IC primers were designed, which incorporated IS900 specific primers that would co-amplify both the IC and the MAP specific IS900 element all in one RT-PCR reaction with one primer set. The IC was cloned into the pMV261 vector by EcoRI and BamHI restriction enzymes and was transformed into the *M. smegmatis* mc2 155 strain. Samples with a combination of both MAP K10 and *M. smegmatis* IC DNA in different DNA concentrations were analyzed by RT-PCR and gel electrophoresis to assess the sensitivity of the IC. Similarly, MAP negative bovine faecal samples were artificially contaminated with a mixture of MAP K10 and *M. smegmatis* IC cells using different ratios of cell concentrations, followed by DNA extractions and RT-PCR analysis to assess the sensitivity of the IC in a bovine faecal environment.

Results: RT-PCR and agarose gel electrophoresis showed that the limit of detection was 4.00 10⁻⁶ ng/μl for both MAP K10 and *M. smegmatis* IC DNA. RT-PCR melting curve analysis yielded distinctive melting curve

peaks corresponding for both MAP K10 and *M. smegmatis* IC when the DNA concentration was 4.00 10⁻⁵ ng/μl, but no distinctive IC melting curve peaks were obtained at higher DNA concentrations than 4.00 10⁻⁵ ng/μl. When the DNA concentration was 10-fold higher for *M. smegmatis* IC compared to MAP K10 DNA, the limit of detection decreased from 4.00 10⁻⁶ ng/μl to 4.00 10⁻⁵ ng/μl for MAP K10. However, melting curve analysis yielded clear distinctive melting curve peaks for both MAP K10 and *M. smegmatis* IC. Similar results were obtained when faecal samples were spiked with a mixture of different ratios of cell concentrations of MAP K10 and *M. smegmatis* IC cells. The detection limit for MAP K10 cells combined with an equal amount of *M. smegmatis* IC cell dilution was as low as 102 CFU/ml for both cultures. Clear distinctive melting curve peaks were obtained for MAP IS900, whereas the melting curve peak obtained for IC was not seen until the cell dilution of 102 CFU/ml for both MAP K10 and *M. smegmatis* IC cells. Increasing the cell concentration of *M. smegmatis* IC cells 10-fold compared to MAP K10 cells resulted in more samples displaying two clear distinctive melting curve peaks corresponding to both IS900 and IC in the same RT-PCR reaction, but MAP sensitivity decreased from 102 to 103 CFU/ml.

Conclusions: By using the *M. smegmatis* strain mc2 155 as a novel internal control model for MAP DNA extractions, it has successfully proven that both MAP IS900 and the IC are successfully co-amplified by RT-PCR in a bovine faecal environment. These results show that the IC can be used to confirm successful DNA extraction and RT-PCR of MAP at low numbers in a faecal environment.

PT1-039-005

Comparative study investigating interference of SICCT on paratuberculosis ELISA in a herd pre- and post-removal of a clinical case.

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Objectives: *Mycobacterium avium* subspecies paratuberculosis (MAP) is the etiological agent of Johne’s disease (JD), a chronic enteritis of ruminants. Administration of tuberculin as part of the single intradermal cervical comparative test (SICCT) for bovine tuberculosis (bTB) has been shown to interfere with MAP ELISA diagnostics. The aim of this study was to investigate the difference in interference of the SICCT test on MAP ELISA diagnostics in a herd pre- and post- removal of a clinical case.

Materials and Methods: A 139-cow dairy herd was recruited to the study. Monthly collection of blood samples for MAP ELISA testing began in May 2012 and continued to December 2014. This included collection of samples pre- (pre-SICCT) and post- (post-SICCT) administration of the compulsory annual SICCT herd test. Additional sampling runs (n=6) were completed up to 90 days post-SICCT in 2012 and 2014 to examine short-term variations in ELISA results. All samples were tested using the ID Screen Paratuberculosis Indirect ELISA (IDVet, France) with results reported as S/P%. Samples recording S/P% ratios of ≥70 S/P% were categorised as positive with all results below this cut-off categorised as negative.

In July 2013, this herd recorded a single cow displaying clinical signs of JD and the presence of the disease was confirmed on post-mortem. A further ten cows which recorded positive MAP ELISA results were culled from the herd and subsequently necropsied in winter 2013.

Multivariable generalised estimating equations (GEE) were used to investigate differences between pre- and post-SICCT S/P% in both 2012 and 2014. Independent variables included sampling time point (pre-SICCT, post-SICCT), parity, calving date, and breed. A Gaussian distribution and an identity link function with an exchangeable correlation was used for analyses. Variables recording P values <0.05 were considered significant. Means and standard errors were calculated for 2012 and 2014 pre- and post-SICCT data. A t-test was completed to investigate whether differences in S/P% between 2012 and 2014 were significant.

Results: All animals tested negative for bovine tuberculosis using SICCT in 2012 and 2014. Following removal of the single clinical JD case, no additional cow recorded pathological lesions indicative of JD. Results from 2012 are outlined in detail in Kennedy et al., 2014. In brief, a pre-SICCT prevalence of 8% increased to 39% post-SICCT, with prevalence returning to pre-SICCT levels by day 71 following administration of tuberculin. In 2014, all cows tested MAP ELISA negative pre-SICCT and had tested negative for four months prior to tuberculin administration, following the culling programme. The proportion of ELISA positives post-SICCT increased to 30%, almost 10% lower than 2012. By day 57 post-SICCT, GEE analysis highlighted no statistical difference between pre- and post-SICCT ELISA readings (P=0.01). It should be noted, however, although S/P% ratios were not statistically different, four cows had S/P% values above the positive cut-off (range 76.7 to 114.5). Only by day 91, did all animals test ELISA negative. Mean S/P% and standard errors from 2012 and 2014 are outlined in the table below and highlights that S/P% are significantly lower in 2014.

	2012	2014	t-test
	Mean S/P% (SE)	Mean S/P% (SE)	P value
Pre-SICCT	26.27 (3.04)	11.46 (0.08)	<0.001
Post-SICCT	44.87 (2.30)	32.43 (1.70)	<0.001

Conclusions: Testing for bovine tuberculosis using the SICCT interferes with MAP ELISA diagnostics in both seronegative and seropositive herds. From the results of the current study, it would appear prudent to avoid MAP ELISA testing of a seronegative herd for a minimum of three months post-SICCT. The overall reduction in S/P% between 2012 and 2014 may reflect the contribution of a MAP positive individual to the anamnestic response in herd mates.

Comments: Aideen E. Kennedy, Ana T. Da Silva, Noel Byrne, Rodney Govender, John MacSharry, Jim O’Mahony, Riona G. Sayers. The Single Intradermal Cervical Comparative Test Interferes with Johne’s Disease ELISA Diagnostics. *Front Immunol.* 2014; 5: 564. doi: 10.3389/fimmu.2014.00564

PT1-039-006

Long term experimental infection of cattle with *Mycobacterium avium* subspecies paratuberculosis (MAP): the first 2 years

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Objectives: *Mycobacterium avium* subspecies paratuberculosis (MAP) causes Johne's disease, a chronic wasting disease of domestic ruminants. The disease has a long subclinical phase before the onset of clinical signs. Currently, it remains challenging to identify the MAP status of subclinically infected cattle. A long term bovine experimental infection study has commenced as part of the IconMAP project, funded by the Irish Department of Agriculture, Food and the Marine (DAFM). Its aim is to search for novel biomarkers of infection status and create a biobank of tissue and blood samples for test evaluation and validation.

Materials and Methods: The experimental infection has been conducted in accordance with the Irish Health Products Regulatory Authority (HPRA) and University College Dublin (UCD) Animal Research Ethics Approval Committee (AREC) requirements. Two low risk farms were identified to source male calves; the calves in the infected group (n=35) received 3.8X10⁹ CFU MAP twice orally at approximately 4 weeks of age, whereas the control calves (n=20) received a placebo. Their MAP infection status is being monitored using currently available diagnostic tests. The IFN- γ assay (Bovigam®) was used to assess the cell mediated immune response against MAP infection. Faecal and tissue samples were cultured for MAP (TREK ESP® para-JEM® system), while the humoral immune response has been evaluated using a commercial ELISA (Idexx). At the month 12 post-infection interval, thirteen animals (n=8 infected; 5 controls) were euthanized for detailed post-mortem examination.

Results: IFN- γ assay (Bovigam®) results are available for months 2, 3, 6, 10, 12, 16, 20 and 24 post-infection and indicate that the experimental infection was successful. Faecal samples from the pre-infection time-point and months 3, 6, 9, 12, 16 and 20 post-infection have been cultured and are negative. Serological results are available from the pre-infection time-point and months 3, 6, 10, 12, 16, 20 and 24 post-infection; a single animal tested positive at the month 10 post-infection interval, two animals tested positive at the month 16 post-infection interval, six animals tested positive at the month 20 post-infection interval and four animals tested positive at the month 24 post-infection interval. At the month 12 post-infection interval, only minor pathological differences were seen between the groups and ileal tissue samples were MAP culture negative.

Conclusions: These results clearly demonstrate the difficulties of identifying subclinically infected cattle using conventional diagnostic tests and emphasize the requirement for novel diagnostic biomarkers of infection status.

PT2-040-001

Transmission of *Mycobacterium Avium* Spp Paratuberculosis; The Role of Faecal Shedding in Dairy Calves

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Objectives: In Johne's disease control, it is generally assumed that calves rarely shed MAP bacteria and that calf-to-calf transmission is of minor importance. Throughout an experimental trial, 61% of the inoculated calves, representing different age and dose groups, shed MAP in the feces at least once. Additionally, we performed a field study on MAP-positive dairy farms and found 2% of young stock MAP culture-

positive with faecal shedding confirmed in all age groups up to 2 years of age. The objective of our follow-up experimental transmission study was to determine if transmission to susceptible penmates occurs.

Materials and Methods: For this study, 32 newborn Holstein-Friesian bull calves were allocated into 8 pens of 4 calves. In 7 of these pens, 2 calves were inoculated with MAP, and 2 calves acted as the contact exposed. The 8th pen was designated as a control group. After initial separation to prevent infections to occur from passive shedding of the oral MAP challenge, the calves were group-housed for 3 months to allow for any potential transmission to occur. After 3 months of group housing, the contact exposed calves were individually housed for another 3 months to allow establishment of infection, whereas the inoculated calves were euthanized. Blood samples for antibody and interferon- γ detection were collected weekly, faecal samples for evidence of MAP shedding were collected three times a week for all animals. Environmental samples of the pens were collected weekly. At the end of the trial all calves were submitted for necropsy to confirm infection in the tissues. The laboratory results will be used to define R0 as well as the transmission parameter β .

Results: Faecal shedding was observed by bacterial culture in all inoculated calves. Although serology and interferon- γ results are still pending, faecal shedding has been confirmed in all contact exposed calves. During the first 3 months of the group housing, between 2 and 10 faecal samples of the contact exposed calves tested positive. Several tissues collected at necropsy of inoculated calves were culture-positive indicating infection from inoculation. The tissue results from the contact-exposed calves are pending.

Conclusions: Based on faecal shedding of MAP, in all 7 MAP challenge groups, transmission seemed to have occurred to contact exposed penmates; pending tissue culture confirmation. This study underlines the importance of calf-to-calf transmission of MAP resulting in infection. JD control programs should implement control measures focused on this route of transmission to achieve a reduction in within-herd prevalence on dairy farms.

PT2-040-002

The diagnostic performance of an antibody ELISA using colostrum to determine the disease status of a Victorian Jersey dairy herd infected with MAP.

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Objectives: Colostrum samples may improve the diagnostic accuracy of some tests when compared to serum for some cattle diseases due to the high concentrations of immunoglobulins present within this sample type. The enzyme-linked immunosorbent assay (ELISA) for Johne's disease is one such test, as it suffers from low sensitivity when testing serum samples collected during the subclinical stage of infection. The aim of the study was to compare the diagnostic performance of a *Mycobacterium avium* subspecies paratuberculosis (MAP) antibody enzyme linked immunosorbent assay with serum or colostrum and using faecal culture as a reference standard.

Materials and Methods: Blood and colostrum samples were collected from 34 Jersey dairy cows from a dairy farm in the State of Victoria, Australia which had previously been confirmed as infected with MAP. The samples were tested for antibodies against MAP by ELISA. Faecal

samples were also collected and tested using a high throughput-Johne's polymerase chain reaction (HT-J PCR) and faecal culture (FC), which was used as the reference test. The diagnostic accuracy (DA) and area under the curve (AUC) were calculated. The HT-J PCR and faecal culture results were also compared.

Results: Of the 34 cows in this study, 4 had FC results consistent with MAP infection. The HT-J PCR did not identify any FC positive cows. Using a 1:20 dilution and S/P ratio cut-off threshold of 0.15, both serum (DA 0.88; AUC 0.44) and colostrum (DA 0.85; AUC 0.37) were unable to identify any of the FC positive cows. With a decrease in sample dilution, serum was still unable to identify any FC positive cows, but colostrum was able to identify 3/4 FC positive cows at a dilution of 1:5 and S/P ratio cut-off threshold of 0.15. The resulting DA and AUC values for colostrum were 0.88 and 0.73, respectively.

Conclusions: In this study, using faecal culture as a reference standard, the diagnostic performance of aMAP antibody ELISA using colostrum was superior to that using serum as well as the HT-J PCR to determine the disease status of a Victorian Jersey dairy herd infected with Johne's disease. Colostrum may provide improved identification of animals in the early stages of infection with MAP. This sample type is easy for farmers to collect and store and the test is relatively inexpensive.

PT2-040-003

Diagnosis of sub-clinical infection with *Mycobacterium avium* subsp. *paratuberculosis* and its effect on milk production

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Objectives: A pilot project to determine if a novel diagnostic strategy coupling multi-antigen serological assay with faecal sampling for enumeration of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) shedding in faeces by quantitative PCR could be used within a commercial 1200 cow dairy herd in South Canterbury, New Zealand to identify and cull animals infected with MAP before clinical disease was evident. Secondary aims were to estimate the association between MAP status as defined by serological and faecal PCR (fPCR) and milk production.

Materials and Methods: In the autumn of 2014 and 2015, a plain coccygeal blood sample was collected from all milking cows in the enrolled herd. The Disease Research Laboratory (DRL), University of Otago, assayed samples for circulating antibody to MAP using two ELISA tests, ParalisaTM and IDEXX. The ParalisaTM test quantifies antibody responses to two distinct protein antigens in parallel, Johnin (PPD-J) and a Protoplasmic Antigen (PPA). An additional MAP-specific recombinant protein antigen, Ag1Del1, was incorporated into the ParalisaTM test and the three tests were read in parallel alongside the IDEXX ELISA assay. For all four serological antigens a classification of Not Detected was returned for results of <50 ELISA Units (EU), readings of 50-100 EU in any one test were low, readings of 101-150 EU as moderate, and readings of >150 EU as high.

Seven days later, a faecal sample (10g approx.) was collected from each cow testing low, moderate or high to any of the ELISA tests and forwarded to DRL for enumeration of MAP shedding in faeces by quantitative fPCR. Faecal sample data were stratified into shedding categories with MAP shedding scores of <103 genomes/ml faeces classified as Not Detected,

scores ≥103-<104 genomes/ml as Moderate and counts ≥104 genomes/ml as High.

The outcome variable was milk solids production (kg milk solids per cow per season). Predictor variables were days in milk, age at sampling in years, ELISA test status and fPCR status. A mixed model with a random intercept for cow and a fixed effect interaction term for year-ELISA gave the model with the lowest Likelihood.

Results: The prevalence of ELISA positive cows was 297/1122 (26.5%, 95%CI=23.9-29.1%) in 2013-14 and 108/1069 (10.2%, 95%CI=8.4-12.0%) in 2014-15 (p<0.001). The prevalence of fPCR positive cows amongst these ELISA positive cows was 55/297 (18.5%, 95%CI=13.9-23.2%) in 2013-14 and 27/108 (25%, 95%CI=16.5-33.0) in 2014-15, (p=0.177).

Milk production was significantly greater in 2014-15 (473kgMS/cow 95%CI=466-479) than 2013-14 (430kgMS/cow (95%CI=424-435), p<0.001). Positive ELISA and faecal PCR were both significant in the final mixed model but there was a significant interaction between year and ELISA status such that the effect of ELISA status was greater in 2013-14 than 2014-15. Across both years the marginal mean production for cows with a high ELISA status was predicted to be 65kgMS/cow less and cows with a low or moderate ELISA status 39kgMS/cow less than cows with a non-detected ELISA status (p<0.001). Cows with a faecal PCR ≥103 genomes/ml faeces produced 46kgMS/year less (p<0.001) than cows with a faecal PCR count <103.

Of the 297 ELISA positive cows in 2013-14, for 2014-15, 122 were culled, 61 were still positive and 114 were ELISA negative. Of the 55 cows testing positive for fPCR in 2013-14, for 2014-15, 52 were culled and 3 were still fPCR positive.

At the observed prevalence of fPCR in this herd, estimation of the sensitivity and specificity of the ELISA test for fPCR status suggest that the ELISA test is very useful for identifying animals that will have an fPCR <103 genomes/ml faeces with a negative predictive value = 92.6% (95%CI=87.2-97.9%).

Conclusions: In the absence of other clinical signs, herd screening using a combined ELISA test for MAP allowed identification of animals with a significantly decreased milk production. ELISA negative animals had a high probability of not being faecal shedders.

PT2-040-004

Experiences with developing a commercial solution to Johne's control in the UK

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Objectives: The control of Johnes Disease (JD) or paratuberculosis in dairy herds is always problematic. The disease has a long incubation with losses occurring in the pre-clinical phase or indirectly as a consequence of increased culling. Effective control depends on education, a clear understanding of the potential economic effects and the enthusiasm of the farmer to implement the changes required to reduce the risk of spread of disease within the herd. In the UK we have sought to find commercial solutions to deliver a National Johnes Management Plan (NJMP) to drive engagement amongst the farmers, vets and wider industry.

Materials and Methods: In 2010 Dairy UK (milk processor representative body) formed a Johnes Action Group to tackle JD in the UK dairy sector.

A collaborative JD engagement programme was developed to engage dairy farmers, milk processors, regional monitoring organisations and veterinary surgeons with the effective control of Johne's disease. The early outputs of the group were focused on establishing coherent messages for inclusion in vet and farmer training, standardised surveillance (targeted 30 cow screen) combined with a commitment to robust risk assessment and management of JD. As a parallel development a commercial web based health planning tool (www.myhealthyherd.com) was designed linking the farmers, vets, monitoring organisations and labs. A specific Infectious Disease manager module within the program facilitated structured risk assessments of disease entry and spread to be completed. A prevalence prediction graph was created using a combination of a targeted milk ELISA sampling of 30 high risk cows and risk assessment results to prompt action from the farmers. The myhealthyherd tool was used to underpin two large regional funded Johnes program and a processor driven education program (Operation Johnes)

In 2015 the engagement plan was enhanced by the development of the National Johnes Management Plan which seeks to engage the processors to encourage their farmers to commit to effective JD control. The first phase of this programme focuses on farmers committing to testing for JD, reviewing their risks and choosing one of 6 control strategies for their farm.

Results: The first 5 years of the programme was viewed as a national "JD engagement" process with the emphasis on education of vets and farmers with 3 main strands of activity; private vets, funded regional schemes and a processor supported farmer engagement scheme (Operation Johnes). Over 2200 farmers completed risk assessments using myhealthyherd.

In the last 5 years over 300 farmer meetings to 2500+ farmers have been delivered with the support of their milk processors with a further unknown number delivered by private vets. Over 350 vets have been educated in the principles of JD control. The farmers and vets were then encouraged to work together to develop an effective JD management plan for their herds using the resources made available.

6 control strategies were developed and accepted within the Johnes Action Group and this has allowed a commercially acceptable solution to be adopted by farmers. The most popular strategies involved improved farm management and testing (50%), improved farm management alone (26%), test and cull (11%) and biosecurity protect and monitor (9%). Vaccination and breed to terminal sire were chosen by less than 2% of farmers. Over 2000 herds now test their herd quarterly for JD.

By September 2016 NJMP has progressed through further financial support from milk processors whereupon 77% of the GB milk supply will be asked to confirm they have tested for presence and assessed the risks of JD within their herds and chosen a suitable control strategy. A webinar 4 stage module of vet training is planned for 2015/16 to create a list of approved JD vets.

Conclusions: Globally well managed voluntary JD programs appears to engage up to 35% of dairy herds. The UK is on target to achieve this level of engagement. The challenge remains as to how well the remaining herds (often of lower prevalence and aspiration) can be successfully engaged with the absence of commercial drivers or compulsion. There is no evidence of a premium to justify risk based trading with many farmers preferring to breed their own replacements. Many challenges remain but with minimal funding and a coherent effort progress can be made to reduce the prevalence of JD within a region or country using commercial approaches.

PT2-040-005

The Thuringian bovine Johne's disease control program – results, experiences and conclusions for the future control strategy

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Objectives: Control of Johne's disease is being discussed controversially, and the eradication of the infectious agent at herd level is disbelieved. Furthermore, prospects to keep herds free from *Mycobacterium avium* ssp. *paratuberculosis* (MAP) or MAP-free and the sustainability of the control efforts are questioned. This study aimed at evaluating the results achieved in voluntary paratuberculosis control measures in cattle herds in Thuringia, federal state of Germany, between 2008 and 2014, and at drawing conclusions for further practice.

Materials and Methods: A total of 76 dairy herds and 29 cow-calf beef herds, keeping a total of 33,614 cattle older than 24 months, enrolled in the voluntary control program and were included into the analysis. MAP shedders were detected by annual testing of cattle older than 24 months by individual faecal culture for *Mycobacterium avium* ssp. *paratuberculosis* (MAP). Only herds with more than 50% of those cattle tested each year were considered. Cumulative incidence (CI) was used to monitor the control progress. A herd was classified as MAP-positive if at least one MAP shedder was detected within the herd and remained MAP-positive until it successfully passed the three-year certification procedure. Herds without any detection of MAP during three consecutive years were certified as MAP-free. Fisher's exact test was used to analyse frequencies of MAP-positive and MAP-free herds relating to their initial MAP status, and a stratified analysis with respect to farm type (dairy or beef) was performed using the Mantel Haenszel test procedure. To analyse the progress of control in dairy herds, a subset of 25 MAP-positive herds was selected. Reduction of CI over the years was analysed using a one-way ANOVA for repeated measurements, and, with respect to the initial cumulative incidence in 2008, using a two-way ANOVA.

Results: In 2014, 27 dairy herds and 13 beef cow-calf herds had been certified as being MAP-free and in seven dairy and six beef herds no MAP shedder was detected and the beginning of the certification period was marked. In three initially MAP-negative herds a reintroduction of the infectious agent was detected during the control period, two of which have been re-certified again. Compared to the MAP-positive herds, MAP-negative herds had a higher chance (odds ratio 32.9; $p < 0.0001$) of achieving a certification as a MAP-free herd until 2014. In 13 of 67 MAP-positive herds (19.4%) the infectious agent was eradicated and the herds got certified as MAP-free. Herds' CI decreased significantly from 2008 ($14.0 \pm 3.6\%$) to 2014 ($5.6\% \pm 1.3\%$) in the subset of 25 MAP-positive dairy herds. Regarding the initial situation in 2008 (CI < 5% or CI > 5%) the control progress differed significantly in a two-way ANOVA.

Conclusions: The results demonstrate that the eradication of paratuberculosis is feasible on herd level. A herd monitoring based on faecal culture tests and a certification period of three years is adequate to justify the status "MAP-free", even if the herd had been MAP-positive in the past. The status "MAP-free" can be maintained by implementing biosecurity and trade controls. Once a herd has achieved a low CI level, lowering the number of new cases per year gets increasingly difficult. Control efforts should be intensified in these herds, e. g. by implementing more strict hygienic measures and by culling the MAP shedders in short term.

PH-028-001

Perception of bovine ringworm by German practicing veterinarians

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Objectives: Bovine ringworm (tinea) is generally being described as a disease regularly occurring in cattle. Actual data are, however, scarce and frequently anecdotal in character. Despite its zoonotic potential, the disease is not being officially controlled in most countries and little is known on the perception of the disease in vets, public or farmers. It was objective of this study to assess the awareness of ringworm by practicing vets and how they judge the perception of the disease by clients.

Materials and Methods: A questionnaire was developed to investigate the perception of practicing veterinarians on ringworm. The areas covered were general assessment of the disease and its zoonotic potential, the perception by farmers, characteristics of the disease and measures against it. The questionnaire was issued to 378 participants before a course in continuing education for practicing veterinarians dealing with the topic of bovine ringworm on 10 occasions. A statement on ringworm could be scored between 1 (no agreement) and 10 (complete agreement). Results were statistically described, an average score was calculated for each question using the formula: (Score*number of participants for this score)/Sum of all scores/Number of participants.

Results: The majority of the participants agreed that ringworm was a serious animal health problem (Agreement score of 7.6) while they generally were neutral about its zoonotic potential being appreciated adequately (Score 5.1). Most participants stated that they diagnosed ringworm on farms regularly (Score 7.3) and that they were actively involved in treating or eradicating the disease (Score 6.8). The participating veterinarians were neutral on the perception of ringworm as animal health problem by the farms themselves as this statement received a score of 5.7. A similar score was calculated on the statement that farms would be actively asking for advice or treatment (5.6). A majority of participants felt that vaccination was helpful and to be recommended (Agreement score of 8.4). The participants were neutral on the statement that the topic had motivated them to visit the course where the survey was done (Score 5.3).

Conclusions: Practising veterinarians feel that bovine ringworm is being perceived less important by farmers than by themselves. Veterinarians judge that the zoonotic potential is underestimated. While vets seem to encounter the disease regularly they apparently are not active in treating or preventing the disease as much. It is to be concluded that more information and offers on the treatment of ringworm by veterinarians could contribute to engaging in the disease more effectively.

Objectives: For animal disease surveillance, in targeted sampling specific herds or animals are selected for sampling due to specific features. For the control of Bovine viral diarrhoea (BVD), testing of a group of young stock is a common method. However, on-farm sampling of young stock is often labour-intensive and dangerous. Therefore, taking samples at the slaughterhouse during meat inspection is straight forward, given that animals to be sampled can be easily identified. We present an application to inform the meat inspectors by highlighting animals to be sampled. They assess the application online through the slaughterhouse ERP-system.

Materials and Methods: We developed a Web-based application (RiBeS) that includes an interface between the animal movement database and the abattoirs' ERP-systems. In RiBeS it is possible to define different types of surveillance an early-detection programmes for various diseases or other targets. As in Switzerland blood sampling was previously not done at meat inspection, we conducted a pilot study at four large Swiss abattoirs and tested various sampling schemes. In May 2015 we used RiBeS to take samples for bluetongue surveillance. Since the pilot study in February we developed the application further and will start with large scale sampling for BVD surveillance in February 2016 at the seven largest abattoirs. With these seven abattoirs, we reach about 80% of the cattle slaughtered. However, there are several hundreds of small abattoirs. For a nationwide coverage, these abattoirs are also important. We plan to test the online application in 2016 for the meat inspectors at these abattoirs.

Results: In the pilot study we were able to confirm that taking samples at the abattoir is more effective than sampling on-farm. However, the investigation costs need to be considered and must be balanced against the savings. Obviously, large numbers of samples lead to a faster return of investment. In the pilot study it was possible to take samples from almost all highlighted animals. Blood samples were taken either from heart at inspection of organs or from the carcass (Plexus brachialis). For high frequent sampling, the organisation at meat inspection needs to be modified and additional personal is necessary. In the pilot study and for bluetongue surveillance we took about 4'500 samples. For the bluetongue samples, the rate of non-eligible samples was much lower with RiBeS than in the previous year without, being 0.1% compared to 0.7%.

Conclusions: Targeted sampling at the abattoirs is feasible and economical. However, the investigation costs are high and need to be well balance against the savings. To sample for animal health programmes at the abattoir might be an important function for meat inspectors given the reduction of classical tasks in meat inspection.

PH-028-003

A systematic review and meta-analysis of control methods for non-typhoidal Salmonella pre-harvest in cattle

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Objectives: A systematic review of the effectiveness of interventions to reduce non-typhoidal Salmonella spp. (hereafter referred to as

PH-028-002

RiBeS – an application for targeted sampling of cattle during meat inspection at Swiss abattoirs for animal health surveillance programmes

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Salmonella) prevalence or concentration in beef and pork was undertaken to provide support to risk managers. This work reports the systematic review findings pertaining to pre-harvest cattle.

Materials and Methods: A broad search was conducted in 2 electronic databases, with additional search verification and grey literature searches conducted. Level 1 relevance screening excluded irrelevant citations; level 2 confirmed relevance and categorized studies. At level 3 screening, data were extracted, and intervention categories (non-therapeutic antimicrobials, biosecurity, feed management strategies, probiotics, and vaccination) were descriptively summarized. Meta-analysis (MA) was performed where 2 or more studies investigated the same intervention in comparable populations. The Risk Ratio (RR) was deemed the preferred summary measure of effect where data supported its computation. We used the Grading of Recommendation, Assessment, Development and Evaluation (GRADE) approach endorsed by the Cochrane Collaboration to assess the weight of evidence for each data subgroup.

Results: Seventy-one relevant primary research studies and four risk assessments/stochastic models were captured. Maintenance of a closed herd was significantly associated with reduced odds of Salmonella on-farm in 7 observational studies. Inconsistent effects of hygiene measures were reported across 3 observational studies. Feeding monensin had a non-significant treatment effect on Salmonella shedding in 3 controlled trials (CTs) with 'Moderate' GRADE rating. Three observational studies reported a significantly ($P < 0.05$) protective effect of feeding antimicrobial-supplemented milk replacer on dairy calf fecal Salmonella shedding. Inconsistent findings were reported for feeding distilled brewers' grains ($n = 3$ CTs, 4 observational studies), cottonseed hulls ($n = 3$ observational studies), or feed withdrawal prior to slaughter ($n = 1$ quasi-experiment, 1 observational study). Feeding of probiotics yielded a non-significant MA treatment effect across 2 CTs with a 'Low' GRADE rating. Salmonella vaccines in cattle had inconsistent treatment effects on fecal shedding, and modest magnitude of summary estimate of effect (Median RR = 0.96, 95% CI (0.75, 1.11)) with 'Very low' GRADE rating across 7 CTs. Heterogeneity of treatment effect in the vaccination dataset could reflect differences between bacterins and live vaccines, or possible mis-matches between vaccine and circulating serovars of Salmonella in the study populations.

Conclusions: While each intervention category was reported effective in some settings, biosecurity practises were most consistently effective. Use of GRADE to assess weight of evidence was found helpful in describing the level of confidence in the magnitude of MA summary estimates.

PH-028-004

The prevalence of verocytotoxigenic *E. coli* O157 (VTEC O157) in British cattle populations

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Objectives: One of the objectives of the British *E. coli* O157 in Cattle Survey (BECS) is to generate new estimates for the prevalence of VTEC O157 in cattle destined for slaughter in both Scotland and in England & Wales, during September 2014 to November 2015. These estimates will build on previous cross-sectional surveys conducted in Scotland

and provide the first equivalent national estimate for England & Wales. These estimates are important as human clinical infection with VTEC O157 has been strongly associated with exposure to livestock faeces and previous sequencing work has demonstrated that Scottish cattle and human cases are closely related.

Materials and Methods: Cross-sectional surveys of 160 farms in England and Wales and 110 farms have just been completed (November 2015). In Scotland, the 110 farms are a random sample of those farms previously visited in two Scottish cross sectional surveys (1998/2000 SEERAD and 2002/2004 IPRAVE), while in England and Wales the design is a randomised, structured survey that replicates the original Scottish surveys. Faecal pat samples have been taken to determine the VTEC O157 status of the farm, according to the previous survey protocols i.e. the cattle preferentially sampled have been weaned animals ultimately destined to enter the food chain; 1g of faeces from each pat was tested for VTEC O157 using IMS; and one isolate per positive faecal sample was tested for Vero toxin 1 and 2, using multiplex PCR. The isolates will subsequently be phage-typed and a temporal and spatially appropriate subset will be whole genome sequenced.

Results: The data from these surveys will be used to generate new herd-level and pat-level prevalence estimates for VTEC O157 in cattle, in each of the populations – Scotland and England & Wales – using generalised linear mixed models. In Scotland, these values will be compared to previous estimates generated using similar methodology. The estimates will also enable comparisons between the two populations. Crude preliminary estimates suggest similar herd-level prevalence for Scotland but a difference between Scotland and England & Wales. Questionnaire data collected at the time of sampling will also be analysed as a means of explaining any differences observed.

Conclusions: Cattle are a recognised reservoir for human clinical VTEC O157 infection. Establishing current baseline prevalence estimates in cattle is a vital first step in any investigation of whether cattle shedding can be linked firstly to the observed consistently high rate of sporadic clinical infection of humans with VTEC O157 in Scotland, and secondly to the observed difference in the human clinical infection rate between Scotland and England & Wales. Future analyses of isolates obtained from these surveys will include the current strain composition and comparisons with data from human clinical cases in the same time period (FS101055).

PH-028-005

Farmers' knowledge of the risk of spread of infection from animals to humans and their transmission prevention practices carried out in 2015.

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Objectives: To determine farmers' knowledge of zoonoses and of the risk of infection posed to humans by some common animal exposures. To determine whether (and how often) farmers carry out simple infection prevention activities on their farms. To find out where farmers get information on animal disease, to inform future health promotion activities.

Materials and Methods: This was a survey of people who submitted material to the Department of Agriculture Food and the Marine's (DAFM) Regional Veterinary Laboratories (RVLs) between February and July 2015. Consecutive persons who presented to the RVLs to submit material for examination during the time period of the study were asked by reception staff to complete the survey. A record was kept of the number of people who declined the survey. The questionnaire was designed by veterinary, scientific and medical members of the South East Regional Zoonoses Committee. Questions related to the farmers' knowledge of zoonoses, their knowledge of the transmission modes/risks from animals to humans, consumption of unpasteurised milk, the water source on the farm house and the testing carried out if this water source was a private well. Other questions were designed to investigate the personal protective equipment worn and other measures undertaken, such as hand washing, to reduce the risk of disease transmission. Demographics of the farmers in the survey were gathered as well as ascertaining how they source information regarding zoonoses.

Results: 1044 farmers completed the survey, 198 refused. Of responding dairy farmers, 39% drink unpasteurised milk once a week or more frequently. 90.1% of the dairy farmers who frequently drink unpasteurised milk didn't know or did not answer the question "Might you or a family member get an infection from healthy animals?". Of responding farmers, 58.5% had private well water at home. Only 35.8% of owners of private wells got their water tested yearly, 43.4% of those with private wells got their water tested less frequently than once a year and 18.3% of private well owners have never got their water tested. Over half of farmers, 52.2%, were not aware of the risk of children contracting vomiting and diarrhoea from animals. Slightly over half (50.6% and 52.3%, respectively), were not aware that disease could be contracted from sick poultry or pets. A high percentage of farmers who completed the survey (89.9%) were not aware that infection can be got from apparently healthy animals. The knowledge of the risk to pregnant women of infection from aborting animals and around animals giving birth was high, with 88.4% of farmers being aware that it was unsafe for pregnant women to be in the vicinity of animals giving birth. The most frequently accessed source of information regarding diseases on farms were: veterinarians (n=913), newspapers (n=519), Teagasc (n=501), DAFM (n=385), social media (n=153), agricultural shows (n=130) and medical practitioners (n=57). Most farmers indicated they used more than one source of information.

Conclusions: The survey findings indicate that the level of farmers' knowledge and awareness about the spread of disease from animals to humans is concerning. Further, this survey has identified the need for further education of the farming community to increase awareness of both the potential biohazards present on farms and the practical measures that can be taken to mitigate the risk of zoonosis.

RE1-023-001

Housing condition (indoor versus outdoor) does not affect the performance of activity meters for oestrus detection in dairy cattle

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Objectives: Pedometers and neck mounted activity tags are widely used for oestrus detection. These tags give oestrus alerts when the activity of a cow exceeds a threshold. To perform well, a daily routine in activity patterns could be important. In the Netherlands, dairy cows are housed outdoors from spring until autumn for 6 to 9 hours a day, depending on the weather. This could mean that in this period the routine is disturbed. The objectives of this study were to investigate whether performance of activity tags differed 1) between pedometers and neck mounted activity collars and 2) between housing conditions (partly outdoor or indoors).

Materials and Methods: Two dairy farms with Holstein-Friesian cows were used in the study. Cows were equipped with pedometers (STL) and neck mounted activity collars (STN) (Smarttag Leg and Smarttag Neck, Nedap Livestock Management, the Netherlands). The study started in the autumn, when the cows were housed partly outdoors ('pasture', September - November) and continued during winter, when the cows were kept indoors day and night ('indoors', November - January). Starting from 20 days after calving milk samples for progesterone analyses were taken two times weekly during morning milking. Progesterone concentration was measured using a commercial ELISA kit (Ridgeway Science, Gloucester, UK). Every oestrus alert generated by the activity tags was compared with the progesterone profile. When an activity alert coincided with low progesterone (< 5 ng/ml), this alert was considered to be correct positive (CP), otherwise it was considered false positive (FP). When no oestrus alert was generated during an oestrus period determined by the progesterone profile, it was considered false negative (FN). Sensitivity (SN) was calculated as CP/(CP+FN) and positive predictive value (PPV) was calculated as CP/(CP+FP). Differences in SN and PPV between farms, activity tags (STL or STN) and housing condition (pasture or indoors) were examined using the Chi-square test for homogeneity.

Results: A total of 138 oestrus periods occurred during the pasture period and 95 oestrus periods occurred during the indoors period based on progesterone profiles. Overall sensitivity and PPV of the STL was 77.7% and 88.3% respectively. Overall sensitivity and PPV of the STN was 79.8% and 84.2% respectively. There was no difference in sensitivity and PPV between the tags. Differences between housing conditions were analysed per tag. Sensitivity and PPV did not differ between the pasture period (STL: 75.8% and 92.3%; STN: 82.1% and 87.6%) and the indoors period (STL: 79% and 85.8%; STN: 78.3% and 81.8%). Sensitivity and PPV did not differ between farms for the STL. For the STN no difference was found in sensitivity between farms. PPV of the STN did differ between farms (93.6% versus 79%, P<0.01).

Conclusions: The performance of the STL and STN are comparable. Although during the pasture period the basal activity of cows may differ from day to day, this does not affect the performance of the activity tags. Performance of the tags (i.e. PPV) differed between the two farms. This could mean that farm specific factors such as cow traffic, indoor housing conditions, herd management etc. cause more or less false positive oestrus alerts and therefore affect the performance of the tags in practice. Which factors play a role is an interesting area of further research.

RE1-023-002

Effects Of Hormone Treatment In Dairy Cows During The Early Puerperium On Fertility Parameters

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Objectives: Hormone treatments in the puerperal period are very popular but the effects documented in literature are controversial. In the presented study the effect of administration of PGF2α or gonadotropine releasing hormone up to 14 days post partum in cows with a physiological puerperium (pp) and a disturbed puerperium (dp) was investigated focusing on further puerperal development and fertility.

Materials and Methods: A total number of 305 cows was included in the study. The PGF2α group (pp1: n=65, dp1: n=41) was treated with 25 mg Dinoprost between 11-14 days post partum. The GnRH group (pp2: n=47, dp2: n=50) received 50 µg Gonadorelin and seven days later 25 mg Dinoprost during the same period after parturition. In the control group (pp3: n=48, dp3: n=54) 1 ml 0.9% Saline was administered between 11-14 days post partum. All injections were given intramuscular. A puerperal examination was done at the day of first treatment, between 25-31 days post partum and according to clinical signs. The body condition (bc) was measured by taking the back fat thickness at three defined periods. Milk production was estimated by calculating the mean daily production. For fertility parameters the data about all performed inseminations was utilized.

Results: Neither Dinoprost nor GnRH-treatment had a significant influence on puerperal involution of the uterus. No differences in all investigated fertility parameters were found. The effect of the hormone treatment on puerperal development and fertility was not influenced by milk production, bc or its decrease during early lactation. Cows with a higher bc shortly after calving had significantly less puerperal disturbances. Bc before calving had no effect on puerperal development or fertility. A distinct decrease of the bc early in lactation meant significantly less success in first insemination. Cows with a lower bc between 60-66 days post partum had a worse pregnancy rate.

Conclusions: The results indicate that no effect on puerperal development or fertility can be found in cows treated with Dinoprost or a combination of Gonadorelin and Dinoprost no matter if they showed a physiological or disturbed uterine involution.

RE1-023-003

The use of Fixed Time Artificial Insemination and re-synchrony in Southern Australian dairy herds

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Objectives: The primary objective of the trial was to describe the reproductive performance of cows after fixed time AI with ovulation synchronisation and re-synchrony in Australian dairy herds, and to compare reproductive outcomes with conventional mating programs.

The secondary object was to compare conception rates between a novel intravaginal progesterone device (DIB-V® Boehringer Ingelheim) and another commercially available progesterone device (Eazi-Breed CIDR®, Zoetis).

Materials and Methods: The study was conducted between October 2014 and March 2015 on 2 seasonal calving commercial dairy herds located in the Murray region of Victoria. Herd 1 consisting of 475 dairy breed cows (Jersey, cross-bred and Holstein Friesian) and herd 2 consisted of 242 Holstein Friesian cows.

Within each herd in the dairy trial: group 1- oestrus detection and AI on detected oestrus for 33 days followed by a natural service period; group 2 – fixed time AI at day 0 followed by re-synchrony of all cows prior to ultrasound pregnancy diagnosis at day 30 and fixed time AI of cows diagnosed not pregnant at day 33 then natural mating; group 3- fixed time AI at day 0 followed by oestrus detection and AI on detected oestrus for 33 days then natural mating. An OVSYNCH-56 with progesterone (DIB-V for group 2 and CIDR for group 3) was used to synchronise oestrus for initial FTAI.

All data was entered into Excel (Microsoft Corporation, 2007) prior to analysis. Statistical analyses were performed using Stata 14 (StataCorp, College Station, TX). The following proportions were calculated: 3-week submission rate, 1st service conception rate, 6-week in-calf rate and proportion of cows that conceiving during the mating period. For proportions pooled over both herds in the dairy trial, confidence intervals were calculated for each group from predicted values after fitting the random effects models. Times to conception were analysed by survival analysis with the Cox proportional hazards models using the `-stcox` command in Stata 14. For the Cox models for the dairy trial, farm was fitted as fixed effect.

Results: Across both herds pooled, 1st service conception rates were similar for all 3 groups (47.77% vs. 51.57% vs. 47.75%) but were lower in herd 2 compared to herd 1. There was no difference in 1st service conception rates between the fixed time AI program that used the commercially available progesterone device- CIDR® (group 3) and the program that used the novel progesterone device- DIB-V® (group 2) (OR=1.15, 95% CI= 0.8-1.7, P-value= 0.459).

Across both herds pooled, there were no statistically significant differences in 6-week in-calf rates between the 3 groups (64.13% vs. 72.40% vs. 68.78%, P>0.05) but there was a tendency for group 2 to have a higher 6-week in-calf rate than group 1 (p=0.064).

Proportions of cows conceiving during the mating period did not differ significantly between the 3 groups (P>0.05) but were higher in herd 1 compared to herd 2. Survival analysis showed no difference in the hazard of pregnancy between the 3 groups. The observed median days to conception for cows that conceived were lower for groups 2 and 3 when compared to group 1 (1 day vs. 1 day vs. 10 days, respectively).

Conclusions: The results of this study showed that fixed time AI with ovulation synchrony and resynchrony combined with early ultrasound pregnancy diagnosis can result in similar first service conception rates, six week in calf rates and proportions of cows conceiving during the mating period to from programs which rely on oestrus detection, natural service or a combination of both.

The first service conception rates in groups 2 and 3 were similar showing that comparable conception rates can be achieved when using DIB-V® when compared to the CIDR® progesterone device for fixed time AI programs in Australian dairy cows.

RE1-023-004

Assessment of daily post partum change in proportion of cows positive to a commercially available test instrument designed to indicate endometritis.

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Objectives: As part of a larger study to look at the effect on reproductive outcome at the herd level of testing individual cows 8 to 28 days post partum (pp) for signs of endometritis and subsequent treatment of test positive (MC+) cows with a single intrauterine infusion of 500mg cephapirin (Metriclean™, Virbac) we first sought to assess the proportion of MC+ cows over the period 8 to 28 days pp.

Materials and Methods: A total of 15,336 lactating Dairy cows from 33 seasonally, spring calving herds on the Central Plateau region of New Zealand's North Island (average 465 cows per herd) were enrolled in the study. All cows were less than 29 days pp at time of enrolment. All herds were visited on three separate occasions at 21 day intervals being about 69, 48 and 27 days before the herd's next Planned Start of Mating. All cows greater than 7 days pp at the day of the visit were examined for signs of endometritis using the Metricheck™ device (Simcrotech, Hamilton, New Zealand) which consists of a 500mm stainless steel probe with a 40mm hemispherical rubber/silicon cup attached at one end used to sample the contents of the anterior vagina which is then assessed for pus as an indicator of endometritis). As visits to individual farms were 21 days apart, every cow was examined 8 to 28 days pp (inclusive). Presence of purulent or muco purulent material was considered to be MC+. The proportion of cows that were MC+ was calculated for each of the individual days pp.

Results: Of the 15,336 cows examined 2,721 (17.74%) were determined to be MC+. A scatter plot of proportion of cows MC+ against days pp (average daily n=730) showed little change (a very slight increase) in proportion of MC+ cows over the period 8 days to 18 days pp but a steady decline in proportion of MC+ cows in the period 19 days to 28 days pp. Logistic regression based on a split at 18 days pp showed that the daily change in the odds of being MC+ over the time period 8 days to 18 days pp was OR= 1.016 (0.9993-1.0336), p=0.06 on 8,576 observations. The daily change in the odds of being MC+ over the time period 19 days to 28 days pp was OR=0.9583 (0.9356-0.9815), p=0.00 on 6,760 observations. Although the daily change in the odds of being MC+ is relatively small it does accumulate over time. The odds ratio of a dichotomised analysis comparing the odds of being MC+ of the two groups of cows (8 to 18 days pp) and (19 to 28 days pp) was OR=0.7352 (0.6762-0.7993), p=0.00 on 15,336 observations (8,576 and 6,760).

Conclusions: The best chance of finding MC+ cows will come from making use of the period 8 to 18 days pp and thus require visits to herds at intervals of no longer than 11 days (14 days maybe most practical though). The carry on, larger study attempts to quantify the change in reproductive performance from treating these MC+ cows with intrauterine infusion of cephapirin (Metriclean, Virbac) with the aim to also determine if the change in odds of MC+ is likely to be due to decreased sensitivity of the test or genuine "self cure".

RE2-031-001

Production And Fertility Of Australian Carry-Over Cows

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Objectives: The objectives of this study were to estimate the reproductive and milk production key performance indicators of cows with 'normal' or 'extended' (carry-over) lactation lengths on pasture-based farms in South Australia. Additionally, the life-time production for these cows was modelled using a hypothetical cow with 'normal' or 'extended' lactation length.

Materials and Methods: Data was collected from on-farm recording systems from 5 dairy enterprises. Statistical analyses were carried out using SAS version 9.3 software.

Cows were grouped into two treatment groups, cows with normal lactation length (≤ 305 -day lactation) and cows with extended lactation or carry-over cows (> 305 -day lactation). Milk production was estimated using modified Wood's lactation curve modelling. The effect of lactation length (normal or extended) on reproductive and milk production key performance indicators were estimated using analysis of variance or logistic regression. The reproductive key performance indicators included the inter-calving interval, waiting period, days open, number of inseminations, re-insemination patterns, 100-day submission and 200-day non-in calf rates.

The hypothetic cow produced the average milk yield per day according to her age and stage of lactation including all potential combinations calving interval of 365- (12 months) or 546-days (18 months) for a life in the herd up to her age of 10 years.

Results: A total of 39,170 and 3,712 cow-lactations were normal and extended respectively were included in the analysis. The inter-calving interval, waiting period, days open and number of services and re-insemination patterns differed between cows with normal or extended lactation lengths ($p < 0.001$). Naturally, the inter-calving interval, waiting period and days open were shorter for cows with 'normal' lactation length. The interesting findings include that cows with extended lactations had more inseminations per conception. The 100-day submission rate was higher in cows with normal lactation length and 200-day not-in-calf rate was lower. Interestingly, cows with a normal lactation had a smaller proportion of short returns compared to cows with extended lactations. Contrary, the rate of long returns was 76.5% for extended lactation cows in comparison to 56.2% in normal lactation cows. This may be a true situation or farmers and farm staff may become more sensitive to cows that have extended lactation and aiming to get them pregnant, submit them even if there is a slight suspicion of being in oestrus.

Average daily milk production was 31.5 and 18.7 L for the cows with normal and extended lactation lengths respectively ($p < 0.001$). The lifetime milk production per hypothetical cow decreased with each extended lactation from maximum 76,947 (8 calvings per cow of 10 years of age) to minimum of 48,124 L (4.5 per cow of 10 years of age; $p < 0.001$).

Conclusions: Cows with extended lactation have poorer reproductive and lactation performance than their counterparts with normal lactation lengths. Cows with extended lactation length appear to be less profitable than those with normal lactation length due to their lower levels of milk production per lifetime and poorer reproductive parameters.

RE2-031-002

Results of an investigation into Dairy Herd Bull Breeding Soundness and Management in Tasmania, Australia

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Objectives: While many Australian dairy herds use a significant level of artificial insemination (AI) as part of their reproductive programs, most still rely on some natural service or bull matings in their herd. This project was aimed at identifying the current status of herd bull breeding soundness in the Tasmania dairy herd. Current practices in the management of dairy herd bulls on these properties and how these may be impacting on bull/herd fertility were also examined.

Materials and Methods: Sixteen dairy properties were selected from the Circular Head region in North West Tasmania. No selection criteria were imposed on the farms selected except that they would consent to all bulls used on their property during the breeding season undergoing a veterinary bull breeding soundness evaluation (VBBSE) and that farmers would complete a reproductive/bull management questionnaire relating to the same season.

In the period of 1 to 2 months prior to when the bulls were to be used in the herd all bulls were subjected to a standardised VBBSE on the property. This examination consisted of a general physical examination and a reproductive examination. Semen was examined crush side and a sample of the semen was also fixed and sent to a laboratory for sperm morphological analysis and live dead counts using nigrosin-eosin staining.

Bull results were collated into a report for farmers and bulls classified into three categories;

1. Fail/Cross - Not suitable for joining this mating period.
2. Qualified - Only suitable in multiple sire situations or with stipulations.
3. Pass/Tick - Suitable with no stipulations.

At the time of the testing farmers were also given a questionnaire relating to their herd reproductive management with an emphasis on bull management on their property. These surveys were collected, the data collated and analysed.

Results: There were 221 bulls tested as part of the project. As a result of the VBBSE, 52 (23.5%) of the bulls were assessed as a fail or high risk, a further 58 (26.2%) of the bulls were given a qualified pass. Therefore, as a result of the fertility examinations 110 (49.8%) of the bulls tested were considered at risk of being sub-fertile on the farms involved in the survey.

The reasons or risk factors for bulls failing the VBBSE or receiving a qualified pass were semen morphology and penile problems.

The herd sizes ranged from 180 to 940 milking cows. The average herd size in the project was 436 milking cows. The average percentage of bulls used with the milking herds was 2.74% with a range of 1.3 to 4%. The average percentage of bulls used with the heifers was 6.1% with a range of 1.3 to 12%. Bulls less than 2 years of age were used in 81% of herds. Bulls greater than 6 years of age were used in 19% of herds.

Of the surveyed herds, 94% used some sort of rotation program for their bulls whilst they were in the herd. Only 13% of the properties sampled had their bulls on the farm for greater than 1 month prior to use and 37% of the farms had their bulls arrive less than 2 weeks prior to being used.

Conclusions: This project illustrates that dairy herd bull fertility is an issue and that there are significant numbers of high risk bulls identified in

the study which will only be identified by a VBBSE that includes semen and sperm analysis.

There are several bull management practices which can be improved in the herds studied. These include improving vaccination and trace element programs for bulls, ensuring bulls arrive on farm earlier, the use of bulls 2 to 6 years of age and the correct percentage of bulls used in the milking herd.

RE2-031-003

Accuracy of diagnosing double corpora lutea and twin pregnancy in the first trimester of gestation in dairy cows

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Objectives: The objective of this present study was to measure and evaluate the usefulness of measuring the P4 and bPAG-1 concentrations in cases of single vs. twin pregnancies and one vs. two corpora lutea at the first four months of gestation. We hypothesized that both the number of the corpus luteum and the number of fetus might have an effect on progesterone and pregnancy protein concentrations and we can set up clinically useful threshold levels in order to predict twin gestations.

Materials and Methods: Eighty-four Holstein-Friesian dairy cows were enrolled in this prospective observational clinical trial. Blood was collected at time-point 1: between Days 29 to 42, time-point 2: between Days 57 to 70, time-point 3: between Days 85 to 98, and time-point 4: between Days 113 to 126 of gestation, respectively and bPAG-1 and P4 concentrations were measured. The animal were grouped into three groups: singleton pregnancy with one corpus luteum (CON), singleton pregnancy with double corpora lutea (DCL), twin pregnancy with two corpora lutea (TWIN). All animals calved according to grouping. After ANOVA analysis animals were redistributed either according to number of corpus luteum or according to number of fetus. Blood samples were analyzed using RIA technique. Statistical analysis was carried out using the Minitab software.

Results: Binary logistic regression analyzing serum P4 concentrations differed at time-point 2 compared to baseline level, but the area under the curve (AUC) had low sensitivity. The bPAG-1 concentrations were statistically different at each time-point of gestation. The AUC cut-off values of serum bPAG-1 concentrations were sufficiently sensitive to differentiate between twin gestations from singleton ones. At time-points 3 (cut-off value of 3.4 ng/mL) and 4 (cut-off value of 56.5 ng/mL) statistically significant differences with low sensitivity, high specificity and a high AUC were found.

Conclusions: Based on these results the diagnosis of twin pregnancy using pregnancy protein measurements is clinically insufficient prior Day 85 of gestation, however the ability to confirm the early twin pregnancy diagnosis with bPAG-1 measurements appears to be promising. To achieve high sensitivity, further studies are required.

RE2-031-004

Associations Between Management Practices and Reproductive Performance in Canadian Dairy Herds

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Objectives: Many options are available to dairy producers for managing reproduction in their herd. Reproductive performance varies greatly from farm to farm, and it is unclear if reproduction management plays a major role in this discrepancy. The objective of this study was to identify management practices associated with reproductive performance on Canadian dairy farms.

Materials and Methods: A bilingual questionnaire was distributed online (FluidSurveys, Ottawa, Canada) and by mail to Canadian dairy farmers from March to May 2014 to assess farm management, as well as reproduction management practices. A total of 53 questions covered demographic information, general management on farm, reproduction management, and opinions and perceptions toward reproduction management. To describe their reproduction management practices, respondents were asked to give their percentage of artificial inseminations (AI) based on visual heat detection, timed AI program, or automated activity monitoring (AAM), for first and subsequent AI separately. The main management practice was defined as the practice used for > 50% of AI, or "combined" if no one practice was used for > 50% of AI. Pregnancy rate (PR), insemination rate (IR), and conception risk (CR) for 2013 were extracted from dairy herd information files (DairyComp 305, Valley Ag Software, Tulare, USA). Linear regression models adjusted for confounders were used to identify associations of these reproductive performance measures with farms' characteristics and management practices. Statistical analyses were conducted using SAS 9.3 (SAS Institute, Cary, USA).

Results: Approximately 9,000 invitations to participate to the survey were sent and a total of 833 surveys were completed (response rate: 9%). A total of 346 farms gave access to their herd files. The average (\pm SD) PR, IR, and CR were $17 \pm 4.7\%$, $43 \pm 11.3\%$, and $40 \pm 8.5\%$, respectively. Pregnancy rate ($\% \pm$ SE) was lower in tie stall barns (15.4 ± 0.7) than in free stall (17.6 ± 0.6), in herds inseminating once per day (16.6 ± 0.8) than in herds inseminating twice per day (18.1 ± 0.8), and in herds with < 100 lactating cows (16.2 ± 0.8) than in herds with more than 250 lactating cows (17.8 ± 0.8). Pregnancy rates were not significantly different by main reproduction management practice: visual heat detection (17.4 ± 0.6), TAI (18.4 ± 0.8), AAM (17.1 ± 1.3), or combined practices (18.2 ± 1.0). Insemination rates were lower in herds using visual heat detection (42.1 ± 0.7) than in herds using TAI (47.4 ± 1.1), or combined practices (46.9 ± 1.2), but not significantly different from herds using AAM (44.0 ± 1.6). Conception risks were higher in herds using visual heat detection (42.2 ± 0.6) than in herds using TAI (39.4 ± 0.8), but not significantly different from herds using AAM (39.6 ± 1.3), or combined practices (39.5 ± 1.0). Positive or negative opinions toward different management tools were not associated with reproductive performance.

Conclusions: Reproductive performance measured by PR, IR, and CR was influenced by factors other than the reproduction management program. These findings suggest that different reproductive management programs can be as efficient, and efficiency can be achieved through other general management practices.

RE2-031-005

Survey on the reproductive management practices and performance of large-scale dairy farms in Hungary

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Objectives: The reproductive performance of the Hungarian dairy cattle population has been continuously declining since the early 80's, despite the introduction of numerous new reproductive management techniques (e.g. OvSynch, pedometer). Therefore, the aim of our study was to survey the current reproductive management practices in the Hungarian dairy herds in order to give advice about how to improve their performance.

Materials and Methods: A survey was carried out in 34 large-scale dairy herds from all the statistical regions in Hungary between 22 May and 6 November 2015. Altogether 25,672 cows were surveyed in these herds, which cover 14.6% of the total Hungarian milk recorded Holstein-Friesian cow population. The average herd size was 755 dairy cows (range: 291-2,502) and the average 305-day milk yield was 10,014 kg (range: 8,330-12,541 kg). In each herd a questionnaire was used to collect the data. Firstly, the farm manager and/or the veterinarian were personally interviewed about the reproductive management practices in their herd, and secondly, the relevant data were gathered from the farm management computer programs. Our questions aimed at the number of animals in the different age groups, the herd-level reproductive indices (e.g. services per conception in cows, first service conception rate, etc.), and the reproductive management practices. Open questions were also included in the questionnaire, so respondents could depict the methods applied on their farms. Data and answers were recorded and evaluated by Microsoft Excel® program.

Results: A voluntary waiting period after calving was applied in 26 herds (76.47%), with an average length of 50.23 days (range: 30-80 days). Visual estrus detection (30 herds; 88.24%) was the predominant method of searching for cows in heat, activity monitoring devices were used in 23 herds (67.65%). In 27 farms (79.41%) estrous synchronization was performed, mostly OvSynch (20 herds; 58.82%). In 3 farms (8.82%) sexed semen was used in cows, but only in those showing intensive signs of estrus. Daily milk production was the most prevalent criterion in reproductive culling decisions (32 herds; 94.12%). Pregnancy was diagnosed 1.83 times per week on average, ranging from monthly (2 farms) to daily (4 farms) pregnancy checks. The average time of the first pregnancy check was 35.06 days after insemination (range: 27-60 days), which was performed via ultrasound in 23 herds (67.65%) and by laboratory PAG test in 2 herds (5.88%). Pregnancy diagnosis was confirmed 1.56 times on average (range: 0-3), typically at least at drying-off. In the majority of farms, efforts were made to protect milking cows from heat stress in summer (ventilator: 30 herds [88.24%]; sprinkling: 22 herds [64.71%]).

The average calving interval was 435 days (range: 392-490 days), average first service conception rate was 26.52% (range: 11.26-51.40%), and average services per conception (cows only) was 4.04 (range: 2.56-6.16), respectively. The breeding interval was 31.38 days (range: 22.00-56.03 days), and the proportion of reproductive culling was 31.68% out of all premature disposals (range: 7.57-69.70%), on average.

Conclusions: The results of our survey clearly showed that the stakeholders are willing to invest in order to improve the reproductive performance of their dairy herds. Practical reproductive management guidelines should be set up by experts that are adapted to the Hungarian circumstances, because the reproductive performance is not effectively monitored in many herds. It is not possible to quantify the pregnancy rate by most of the currently used farm management programs, however, it

is a basic parameter. The findings of the survey show that there is room for improving reproduction, thus, the competitiveness in the majority of the Hungarian dairy herds.

RE2-031-006

Time of ovulation relative to onset of oestrus as determined by visual observation and Heatime™ activity meters in loose housed Hereford cattle

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Objectives: The aim of this study was to investigate the time from oestrus detection using both visual and a commercially available heat detection system (Heatime™) to ovulation in housed purebred Hereford cattle.

Materials and Methods: This study was performed at the Götala Beef and Lamb Research Centre, Sweden between 01/06/15 and 03/07/15. The study consisted of 37 purebred female Hereford beef cows (14 nulliparous 23 pluriparous) which were housed in loose housing on deep straw bedding. The primiparous animals were all aged between 13 and 15 months at the start of the experiment and had a mean weight of 407 Kg. The animals had a modal body condition score of 3.5 (range 2.5 to 4.0).

Blood samples were taken thrice weekly from all study animals. Serum was extracted, frozen and analysed for progesterone concentration using an ELISA (Ridgeway Scientific UK). Luteal activity was defined progesterone concentration >1 ng/ml.

Visual observation of oestrus was performed for 20 minutes thrice daily at 0600, 1400, and 2200. When a behaviour associated with oestrus was observed a score was assigned to that cow, when the scores summated to 100 or more the cows were deemed to be in oestrus (Van Vliet, 1996). A commercially available oestrus detection system Heatime was according to the manufacturer's guidelines.

Once oestrus was detected cows reproductive tracts were examined thrice daily (after the oestrus observation period) using real time ultrasonography to determine the time of ovulation.

Results: Oestrus was not detected in 8 (4 nulliparous and 4 multiparous) animals. The progesterone concentration in these animals did not rise above 1 ng/ml for two consecutive progesterone measurements and were therefore deemed to be in anoestrus throughout the study period.

In total there were 46 oestrus events with recorded ovulations (40 visually detected oestrus and 46 detected with Heatime™). There were 40 visually detected oestrus with determinable ovulation. The mean time from visual oestrus detection to ovulation was 19.7 hours (95% confidence interval 15.8 – 23.6 hours). In nulliparous animals (n=16) the mean visual oestrus to ovulation interval was 16.8 hours (8.6-24.9 hours). Whilst in multiparous animals (n=24) the mean visual oestrus to ovulation interval was 21.7 hours (17.7 - 25.6 hours).

There were 46 oestruses with determinable ovulation detected by Heatime™. The mean time from visual oestrus detection to ovulation was 21.6 hours (95% confidence interval 18.1 – 25.0 hours). In nulliparous animals (n=16) the mean visual oestrus to ovulation interval was 21.3 hours (15.7 - 26.8 hours). Whilst in multiparous animals (n=30) the mean visual oestrus to ovulation interval was 21.7 hours (17.2 - 26.3 hours).

Conclusions: Whether visual oestrus detection or Heatime™ oestrus detection is used there is no practically important difference in time from oestrus detection to ovulation. When either method of oestrus detection is used nulliparous animals had a numerically shorter interval from oestrus detection to ovulation. However, the difference is so small that practically the same protocol should be used irrespective of whether the animal to be inseminated is nulliparous or multiparous

RE2-031-007

Effect of GnRH administration at initiation and eCG at the end of a 7 Day-Co Synch+progesterone program for FTAI on pregnancy rate in beef cows

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Objectives: The objective of this study was to compare the effectiveness of three different oestrous synchronisation protocols to facilitate fixed time artificial insemination (FTAI) in suckler beef cows under Irish pasture-based field conditions.

Materials and Methods: A total of 1410 suckled cows located on 61 farms were enrolled across two replicates (in Spring, S, n=703 and Autumn, A, n=707). After a voluntary waiting period of 35 days postpartum, all cows were ultrasound scanned to determine the presence or absence of a corpus luteum (CL) and body condition score (BCS) was recorded on a scale of 1 to 5. Cows were then randomly assigned to receive a 7 day progesterone-releasing intravaginal device (PRID E®) without (Group 1) or with (Group 2) a GnRH analogue (Ovarelin®) at PRID insertion and a luteolytic dose of prostaglandin F2α (Enzaprost®) at PRID removal. A third group of cows received 400 IU equine chorionic gonadotropin (eCG, Syncrostim®, Group 3) at PRID removal.

After 72 h from the PRID removal GnRH was administered and all cows were inseminated. Pregnancy diagnosis was carried out 35-40 days after FTAI by transrectal ultrasonography. Data were analyzed using the GENMOD procedure of SAS.

Results: There was a significant treatment by season interaction for pregnancy rate (P=0.0002). In Spring, overall pregnancy rate was 59.1% (416/703) and was affected by treatment (49.6 v 59.3 v 68.5%, for Groups 1, 2 and 3, respectively P<0.05). In contrast, in Autumn, overall pregnancy rate (52.6%, 364/707) was unaffected by treatment (53.7 v 52.0 v 48.7%, respectively). Presence of a corpus luteum at initiation of treatment (S 50.6%; F 70.0%) increased pregnancy rate independently of treatment and season (P=0.03). The mean BCS in S=2.75 and A=2.92 was not different, but BCS positively affected pregnancy rate independently of treatment or season (P=0.003).

Conclusions: The administration of GnRH at the initiation of a 7 day Co-Synch+PRID E protocol and the supplementation of 400 IU of eCG at PRID E removal, both increased pregnancy rate in Spring calving suckled beef cows. This effect was not evident in Autumn calving cows. Seasonal differences in outcome may be reflective of different managements (grazing v confinement), interval post-partum AI or breed type and remain to be elucidated.

SR1-020-001

Development of an ovine model of digital dermatitis infectionJarlath Nally^{1,*}, Jennifer Wilson-Welder¹, David Alt¹, Paul Plummer²¹Infectious Bacterial Diseases, USDA, ²Veterinary Diagnostic and Production Medicine, Iowa State University, Ames, IA 50010, United States

Objectives: Digital dermatitis is an infectious cause of lameness primarily affecting dairy cattle, but also beef cattle, sheep, goats, and more recently, in a small population of North American wild elk. Digital dermatitis is associated with a mixed bacterial infection, including several *Treponema* species, and other aerobic and anaerobic bacterial species. Whilst the exact etiology has not been demonstrated, a number of bacterial, host and environmental factors contribute to disease development and progression. In order to study host-bacterial interactions and develop effective therapeutics, a reproducible laboratory model of infection is required.

Materials and Methods: Crossbred sheep of mixed ages were obtained from a breeding flock free of hoof disease. Hind feet were abraded to create a superficial wound before wrapping to emulate a moist, anaerobic environment, similar to that believed to predispose animals to infection. After 3 days, wounds were inoculated once (in a final volume of 1ml) to macerated lesion material from active cases of bovine digital dermatitis and remained wrapped, with periodic rewetting. Animals were monitored for lameness and lesion development for several weeks.

Results: Experimentally inoculated hind feet developed lesions indicative of infection by two weeks post-infection. At this time, swabs of lesions were positive for the presence of spirochetes as observed by dark-field microscopy. At four weeks post-infection, histologic changes in the dermis and epidermis were consistent with those described for bovine digital dermatitis, including erosion of the keratin layer, ballooning of underlying keratinocytes and neutrophil dominated inflammatory infiltrate. Silver staining of lesion biopsies at four weeks confirmed that spirochetes penetrated host tissue. Biopsied material was subsequently used to reproduce and maintain infection in naïve sheep, predisposed as described above.

Conclusions: Digital dermatitis is an infectious disease that can be reproduced in experimentally infected sheep. The continued development and refinement of this ovine model of digital dermatitis will allow for novel insights into pathogenic mechanisms of infection, as well as the development of improved diagnostic and therapeutics.

SR1-020-002

Prevalence, clinical findings and progression of extensive fibrinous pleurisy in adult sheep based upon ultrasonographic examination of the chest.Phil Scott^{1,*}¹Capital Veterinary Services, Haddington, United Kingdom

Objectives: The objective of this study was to investigate the prevalence, clinical findings and progression of fibrinous pleurisy lesions in adult sheep in the United Kingdom following initial ultrasonographic diagnosis.

Materials and Methods: Fibrinous pleurisy in sheep cannot be reliably diagnosed on auscultation of adventitious sounds alone therefore the

initial screening process involved examination of both sides of the chest using a 6.5 MHz microconvex probe connected to a real-time, B-mode ultrasound machine. All adult sheep in four flocks (comprising 350, 500, 700 and 850 breeding ewes, respectively) screened for ovine pulmonary adenocarcinoma (OPA) during autumn 2015 were also examined for fibrinous pleurisy. Sixty five adult sheep presented to the University of Edinburgh Veterinary School's Farm Animal Hospital (FAH) over a two years' period 2013-5, where the cause of illness was not readily established, were scanned for lesions of the lungs/pleurae.

Results: Two sheep with extensive unilateral fibrinous pleurisy were identified in the 700 ewe flock during the OPA screen; neither sheep showed signs of illness. Three sheep with fibrinous pleurisy were diagnosed ultrasonographically in the FAH and presented with chronic weight loss (2) while one ewe presented with an increased respiratory rate, abdominal breathing and weight loss. Auscultation in all cases revealed absent normal breath sounds and greatly reduced heart sounds on the affected side of the chest with increased wheezes and louder heart sounds on the unaffected side. No pleuritic rubs were heard. Procaine penicillin administration daily for four weeks coincided with rapid resolution of lesions within two weeks except for one ewe where the pleurisy extended to 15 cm and appeared well encapsulated. The extensive fibrinous exudate extending >8 and 10 cm in two recovered sheep appeared as discrete 3-4 cm spherical hypochoic areas when re-scanned two weeks after treatment commenced and these lesions could not be differentiated ultrasonographically from an abscess.

Conclusions: This is the first report of ultrasonographic monitoring of an extensive fibrinous exudate within the pleural space of sheep which was not detected during thoracic auscultation. None of the six sheep was sick nor showed signs of acute respiratory disease. The efficacy of antibiotic therapy could not be assessed because no animal was left untreated but the rapid resolution of extensive fibrinous exudate in three sheep would suggest some beneficial effect. The discrete spherical hypochoic area in the pleural space observed two weeks after initial diagnosis could not be differentiated ultrasonographically from an abscess.

SR1-020-003

The impact of infection with Schmallenberg virus on weaning rate in Irish sheep flocksDamien Barrett^{1,*}, Donal Sammin², Ronan O'Neill², Tracy Clegg³, Simon More³¹Sligo RVL, Dept of Agriculture, Food and the Marine, Sligo, ²Central Veterinary Laboratory, Dept of Agriculture, Food and the Marine, ³Centre for Veterinary Epidemiology and Risk Analysis, University College Dublin, Dublin, Ireland

Objectives: In Ireland, SBV was subsequently confirmed by RT-PCR in 49 cattle herds and 39 sheep flocks in 2012 and 2013. While these studies provide a good representation of the spatial distribution of SBV in Ireland, they do not quantify the impact of SBV on productivity. The objectives of this study were to assess the impact of SBV on weaning rate in Irish sheep flocks, based on data reported by Irish sheep farmers, and to evaluate weaning rate in sheep flocks as an indicator to be used in emerging disease surveillance systems.

Materials and Methods: A questionnaire on productivity and management practices in sheep flocks was developed. These questionnaires were distributed among sheep farmers where SBV had been confirmed and through farmer discussion groups. Valid responses from 267 sheep farmers were received. Excel 2007 (Microsoft, USA) and

transferred into Stata SE 12 (Statacorp, USA) for analysis. Univariate and multivariate negative binomial regression model was carried out on the data. Maps were created using ESRI Arcview 3.2 (Redlands, California), with flock location determined using the geo-coordinates maintained by the DAFM national herd database.

Results: Group 1 flocks (with a laboratory confirmed case of SBV) had a weaning rate approximately 90% that of the control Group 4 flocks (negative flocks in a county where SBV had not been diagnosed). The weaning rate among mountain breeds of sheep was approximately 90% that of lowland breeds, where there was a mixture of lowland and mountain sheep, the weaning rate was approximately 95% that of lowland sheep. For each unit increase in flock ewe barren rate, the weaning rate was 0.39 times lower than that of a flock with no barren ewes. Similarly, for each unit increase in 2012 flock weaning rate, the flock 2013 weaning rate was 1.65 times greater. Examination of the residuals indicated that there was no significant lack of fit in the final model and no significant outliers.

Conclusions: The 10% reduction in weaning rates as a result of where the presence of SBV was confirmed is a justifiable concern for sheep farmers. Several other factors relating to management and breeding were also found to significantly affect weaning rate. As there was no pre-existing production database in existence, the data had to be collected by questionnaire. In a broader context, this study shows the value of interrogating production data in conjunction with an animal disease database in estimating the economic impact of an emerging disease.

SR1-020-004

A study on tuberculosis in camelids in Al-Muthanna governorate /Iraq

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Objectives: There is a total of 58,000 camels in Iraq, according to the FAO statistics of 2011 (Tara, 2011). The extent of TB has been well documented in humans and most domestic animals in Iraq and according to the latest WHO data published in May 2014 Tuberculosis deaths in Iraq reached 928 or 0.63% of total deaths. However, very little is known about the epidemiology and cause of camel TB in Iraq. Therefore, this study was intended to investigate the epidemiology of camel TB based on gross pathology and identify its causative agents using mycobacteriological culturing, and multiplex polymerase chain reaction (PCR) in Al-Muthanna governorate in Iraq.

Materials and Methods: A cross sectional study was conducted on 50 apparently healthy camels male (n=25) and female (n=25) slaughtered at Al Muthanna abattoir. For each animal, post-mortem examination was performed according to routine procedures. Mandibular, retropharyngeal, bronchial, mediastinal, mesenteric and hepatic lymph nodes were examined. Lungs, liver, small intestine and kidneys were also examined in detail. The suspected lesions on both left and right lungs were sectioned, inspected and palpated carefully. Whenever gross lesions suggestive of TB were detected in any of the tissue, the tissue was classified as having lesions. For mycobacteriological culturing, samples were cultured on Lowenstein Jensen (LJ) medium on duplicate slants, one enriched with sodium pyruvate and the other enriched with glycerol. Cultures were incubated aerobically at 37°C for 8–12 weeks with weekly observation for growth of colonies. Positive cultures were confirmed with Ziehl Nelsen staining and preserved with freezing media. The multiplex

PCR differentiate *M. tuberculosis* complex from *M. avium* complex, *M. intracellulare* and other mycobacterial species. Multiplex polymerase chain reaction (PCR) was done for all isolated bacteria.

Results: The distribution of lesions and the severity of the disease were established in the 24 camels with suspicious lesions. The TB lesions were seen in the lymph nodes and lung lobes of the examined animals. On the basis of gross pathology, the prevalence of camel TB was 48 % (24/50). The mycobacterial growth was confirmed in 50 % (12/24) of the samples collected from camels with grossly suspicious TB lesions. Mycobacterium genus typing was conducted on the 12 culture isolates from camels. Based on multiplex PCR using the primers of the *M. tuberculosis* complex and *M. avium* complex, 9 isolates gave signal to the genus *Mycobacterium*.

Conclusions: The results of the present study have shown that the camel TB lesions are caused by mycobacteria other than *Mycobacterium tuberculosis* complex. The author, hence recommended further identification and characterization of these species, which would be useful towards the efforts made to control TB in camels.

SR2-021-001

Sheep And Farm Level Factors Associated With Contagious Ovine Digital Dermatitis: A Longitudinal Repeated Cross-Sectional Study Of Sheep On Six Farms

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Objectives: Contagious ovine digital dermatitis (CODD) is a cause of severe lameness in sheep in the UK currently affecting approximately 50% of farms. The epidemiology is poorly characterised and there have been no on farm studies investigating risk factors for CODD. The aims of this study were to investigate 1) the prevalence dynamics of CODD, 2) the association between sheep with CODD and potential risk factors and 3) the impact of CODD on lameness in sheep.

Materials and Methods: Six farms with CODD were studied in North Wales. The farms were visited at two-month intervals between June 2012 and October 2013. At each visit all lame sheep and randomly selected non-lame sheep were examined (ratio 1-3) resulting in a total of 6,515 sheep examined. Descriptive statistics, together with logistic regression modelling techniques employing probability weights were used to describe the prevalence of CODD within and between farms and to investigate temporal variations as well as farm, environment and sheep level risk factors associated with CODD.

Results: The prevalence of CODD varied between farms (2.5-11.9%). Within farms, prevalence may increase in the early autumn and after housing. Environmental risk factors included poached pasture. Co-infection with footrot was strongly associated with CODD OR: 7.7 (95%CI: 3.9-15.5 P<0.001) but negatively associated with co-infection with interdigital dermatitis (ID) OR: 0.04 (95%CI: 0.02-0.1 P<0.001). Reinfection with CODD was observed in 78 individuals but there was no re-infection at foot level.

Lameness on all farms reduced during the study and seasonal changes in lameness followed similar patterns to those for CODD. Infection with CODD leads to a greater increase in locomotion score compared to footrot or ID and CODD lesion grade was strongly associated with being lame. Sheep with CODD in more than one foot were more likely to be

lame OR: 25.0 (95%CI: 12.5-49.9 P<0.001) than those with just one foot affected OR: 10.0 (95%CI: 8.6-11.6 P<0.001).

Conclusions: The biggest risk factor for CODD was co-infection with footrot and thus control of footrot may help reduce the risk of CODD on affected farms. Environmental risk factors for CODD were similar to those for footrot adding weight to control strategies that target both diseases in tandem. This disease whilst currently identified only in the UK and Ireland causes severe welfare implications for affected sheep. This paper highlights key risk factors which may assist in developing control strategies.

SR2-021-002

Efficacy of Chitosan, a Natural Polysaccharide, against *Cryptosporidium Parvum* Infection in Vitro and in Vivo in Small Ruminants

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Objectives: Cryptosporidiosis is a zoonotic disease caused by a protozoan parasite, *Cryptosporidium parvum*. In animals, it is considered as an economically important disease with clinical signs and death in young ruminants. The usual clinical course is acute diarrhoea affecting animals from 1 to 3 weeks old. Today, no drugs are fully effective in the treatment of cryptosporidiosis in man and animals. Therefore the research for new therapeutic agents is crucial.

Materials and Methods: We report here details of the adaptation of in vitro culture systems (HCT-8 and Caco-2 cell lines) for *C. parvum* to investigate the "anticryptosporidial" activity of drugs and the results obtained with two new molecules (Chitosan NAG and Chitosan Mix).

Chitosan is a sugar that is obtained from the hard outer skeleton of shellfish, including crab and shrimp. It is used for medicine. Chitosan, a natural polysaccharide compound, has been found to be active against a variety of diseases including antimicrobial and antitumoral effects. We investigated the effects of Chitosan in our two in vitro models we established in the laboratory. Paromomycin, a classical drug used in veterinary medicine, was used as a positive control. Immunofluorescence technique was used for the identification and enumeration of the parasites.

Results: Our results showed a very significant reduction of viability of *Cryptosporidium* oocysts (>95%) (in comparison with non treated samples) after pre-incubation of 24h at 37°C with Paromomycin (P < 0.001), Chitosan Mix and Chitosan NAG (P < 0.001). On the other hand, Paromomycin, Chitosan Mix and Chitosan NAG inhibited significantly the development of *Cryptosporidium parvum* in HCT-8 and Caco-2 cell lines (P < 0.005). These effects are dose-dependent. Synergic effects were obtained when Chitosan NAG treatment was associated with Paromomycin.

Conclusions: In conclusion, these findings provide for the first time the evidence of in vitro inhibitory activities of natural polysaccharides against *C. parvum*. The efficacy of Chitosan was evaluated in goat neonates experimentally inoculated with *Cryptosporidium parvum* oocysts per oral route. The preliminary results showed the efficacy of chitosan in reducing oocyst shedding and diarrhoea in goat kids cryptosporidiosis.

SR2-021-003

An outbreak of *Anaplasma phagocytophilum* in a goat herd in the Province of Trento (North-eastern Italian Alps): a case report

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Objectives: *Anaplasma phagocytophilum* (AP) is an emerging tick-borne bacterium with both veterinary and human health implications. AP is spread over a wide territory of the USA, Europe and Asia vectored by ticks of the genus *Ixodes*. The disease in domestic ruminants, also called tick-borne fever (TBF), has been recorded most frequently in cattle and sheep; while poor data on clinical cases of TBF associated with naturally acquired infection is available in goats. This case report describes the first evidence of a severe AP outbreak in naturally infected goats in the Province of Trento (North-eastern Italian Alps).

Materials and Methods: In June 2015 the farm veterinarian reported a febrile syndrome in five lactating goats at pasture characterized by high temperature (>40°C), apathy, poor appetite, sudden drop in milk production associated with an increase of the somatic cell count and hyperaemic stains on udder skin. Within the following 3 weeks approximately 90/200 lactating goats (45%) showed similar symptoms. Most of the affected animals recovered spontaneously in 2-4 days without therapy; 10 of them presented diarrhoea and 4, among the older ones, died. Clinical signs appeared only in grazing lactating goats and the farmer reported that 4-5 ticks were removed per goat daily. For the first diagnosis whole blood samples and sera of 2 recovered and 3 symptomatic animals were tested for haemoparasites (AP, *Babesia* spp. and *Theileria* spp.) using PCR assays (Massung & Slater, 2003; Casati et al., 2006) and for AP antibodies by an immunofluorescence assay (IFAT) using the cut-off titre of 1:80 for both IgG and IgM. After the outbreak, 22 adult goats were randomly selected to investigate patterns of AP infection within the herd. Animals were kept under observation for clinical signs and molecular and serological investigations on blood samples, collected in July and in September 2015, were performed. On September 2015 questing ticks (73 nymphs and 9 larvae) were also collected from the pasture by standard blanket dragging. The ticks were pooled into pools of five and molecularly examined for AP. Some representative positive samples were sequenced to perform a phylogenetic tree.

Results: Molecular investigations revealed the presence of AP in each goat tested for the first diagnosis and only one showed specific IgG antibodies (1:80). In July 8/20 (40%) goats tested positive for AP in blood samples by PCR and 15/20 (75%) had IgG antibodies against AP with titre ranging from 1:160 to 1:2560. In September only 3/22 (9%) animals resulted positive for AP by PCR and 9/22 (41%) goats resulted positive for IgG antibodies against AP with titre ranging from 1:160 to 1:5120. Among 8/20 goats positive for AP, 4 heads showed drop in milk production, 3 have had symptoms in July and 1 was apparently healthy; at the second sampling only one animal tested positive again by PCR and 7 goats became negative. Antibody titres showed a progressive decline in each seropositive animal but 3 heads: one goat developed antibodies and 2 presented an increase in antibody titre at the second sampling. Questing ticks collected at the pasture were identified as *Ixodes ricinus* and 2 pool of nymphs resulted positive for AP, showing a minimum infection rate of 2.4%. However the tick infection rate at the time of transmission remains unknown. The sequences obtained from blood and tick samples were identical to each other and clustered with the Italian, Polish, American and Japanese strains (KJ663729, EF143803, JQ063013 and FJ600591).

Conclusions: To our knowledge this report is the first case of a severe AP outbreak in naturally infected goats. The high number of animals with clinical manifestations in the same herd was unexpected and has led to significant economic losses. Since an iatrogenic transmission was excluded, our findings suggest a focus of a highly pathogenic AP strain or an outbreak characterized by a high infection rate in ticks and in wildlife. Further investigations in this goat herd are ongoing in order to study the bacteraemia and antibody dynamics over time. The results will give new information on possible chronic infections and clinical relapses in goats.

SR2-021-004

Staphylococcus aureus in Dairy Goats and Human Milkers in North Carolina (USA)

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Objectives: The objective was to determine the prevalence of *Staphylococcus aureus* in aseptically collected milk samples and nasal swabs from lactating dairy goats and from the nose and hands of individuals milking the goats. We determined the % presence of *S. aureus* in samples from the various sites and compared antibiotic susceptibilities and genotypes of the *S. aureus* isolates. Herd characteristics and management practices were obtained from surveys completed on each dairy.

Materials and Methods: Samples were collected (September of 2014 to July of 2015) from 30 NC properties with dairy goats. The total included 519 milk samples, 502 goat nasal swabs, and 97 human swabs. Dairy goats were found on properties that ranged from small backyard to larger commercial operations. Aseptically collected milk and nasal swab samples were obtained from all milking does. Hand and nasal swab samples were collected from individuals milking the goats. For each herd, data was collected on animal numbers and on the herd's milking and mastitis control practices.

Milk samples (0.1 ml) were plated and bacteria present identified using procedures consistent with NMC recommendations. Swab samples were macerated in 0.85% sterile saline and 1 ml plated on 3M Staph Express Petrifilm plates according to manufacturer's instructions. Antibiotic susceptibilities for the *S. aureus* isolates were determined using the Kirby-Bauer method according to Clinical Laboratory Standards Institute (CLSI) protocol. Genotyping was accomplished by PFGE using US Centers for Disease Control (CDC) protocols with minor modifications, and PFGE gels were analyzed and dendrograms constructed using BioNumerics software.

Results: A major finding of the study was that *S. aureus* was infrequently a cause of IMI (1.2% of all milk samples) in the NC herds studied. In contrast, nearly half (46.2%) of all goat nasal swabs were *S. aureus* positive. Approximately one-fourth of human nasal and hand swabs were positive for *S. aureus* (25.8%). Preliminary PFGE analysis reveals some sharing of *S. aureus* genotypes between goats and humans and possibly other animals. Some isolates of *S. aureus* from goats displayed evidence of multi-drug antimicrobial resistance.

Surveys indicated that most goat owners consumed raw milk from their goats. Some owners made cheese from the milk. Most farms milked their goats twice a day, either by machine, by hand, or both. Only rarely did producers report wearing gloves during the milking process. A variety of procedures were used in pre-milking preparation, but all producers used post-milking germicidal teat dips/sprays.

Conclusions: *Staphylococcus aureus* was infrequently (1.2%) isolated from milk samples of NC dairy goats, but was commonly detected (46.2%) in goat nasal swabs. Only some goats with *S. aureus* displayed multi-agent antimicrobial resistance. Goat owners appear to be successful in minimizing the presence of *S. aureus* as a cause of IMI.

SR2-021-005

Comparative effects of Border Disease Virus genotypes on ovine fetal infection

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Objectives: Border Disease is a worldwide disease of sheep caused by Border Disease Virus (BDV). With 7 phylogenetic groups, the BDV genetic diversity is large but its impact on pathogenesis is not well understood. In France four BDV genotypes, 3, 4, 5 and 6, have been identified since the first description of BD in 1984. Only BDV-3 and BDV-6 genotypes seem to be responsible for a recent French severe epizootic. The objectives of this study were to compare, in a same ovine model, the pathogenesis of the four French BDV genotypes. Fetal infections, ability to induce abortions, fetal malformations and production of permanent infected lambs were recorded.

Materials and Methods: Animal experimentation was performed under EEC guidelines (86/609/CEE) and official French ethical agreement. Four groups of 8 pregnant ewes at 52 days of gestation and free of pestivirus (negatives for both antibodies and BDV) were inoculated (Day 0) by the intramuscular route with 2.105 TCID₅₀/animal of BDV-3, BDV-4, BDV-5 and BDV-6 genotypes, respectively. BDV-5 AV was the first strain isolated in France in 1984. BDV-3 10405 and BDV-6 6390 strains were isolated in 2010 from recent French BD epizootic. BDV-4 IZ strain was isolated from an izard (*Rupicapra pyrenaica*) and recently detected in French Pyreneans sheep. A fifth control group was not infected. Complete clinical examinations of ewes were recorded daily from 3 days before inoculation (D-3) to D18 post-infection and for abortion until the end of the experimentation. Haematological examination was performed every other day from D-3 to D18 using a Melet Schloesing 9-5 analyzer. Viral infection of ewes was examined in blood EDTA samples from D-1 to D18 by real-time reverse transcription-PCR (RT-qPCR, Taqvet pestivirus, Life Technologies), and antibody response kinetics was measured by NS2-3 ELISA (Life technologies) and seroneutralisation every week until D64. The ewes were euthanized two months after challenge (D64) at 117 days of pregnancy. Fetuses were immediately collected, examined for viability, malformations, growth retardation (atlas-tail base, radius and tibia lengths), antibody status (NS2-3 ELISA and neutralisation assays) and BDV presence in the brain, thymus and spleen (RT-qPCR, Life Technologies).

Results: No clinical signs were observed in ewes after infection except in the BDV-5 group which showed moderate depression at D8 and D9 (all animals). Infections with each genotype were followed by a significant transient leucopenia from D3 to D7, mainly characterized by lymphopenia. Lymphopenia was more severe for the BDV-4, BDV-5 and BDV-6 groups but with no significant differences (Anova) when compared with BDV-3 group. Virus could be detected in 5, 6, 8, and 5 sheep of BDV-3, BDV-4, BDV-5 and BDV-6 groups, respectively with

a mean duration of 1.2, 1.4, 4.2, and 1.4 days, respectively. Abortion was observed only in two BDV-5 inoculated sheep. At the end of the experiment, 14, 17, 20 and 12 fetuses were collected in the BDV-3, BDV-4, BDV-5 and BDV-6 groups. Large differences were observed for gross examination of fetuses. In the BDV-5 group, 62.5% of fetuses died and 8.3% of live fetuses had malformations (arthrogryposis, hydrocephalus, cerebellar atrophy). The BDV-4 and BDV-6 groups contained only viable fetuses but with malformations for 58.8% and 10% of them, respectively. Neither lethality nor malformations were found in the BDV-3 group. In contrast BDV loads (as measured by RT-qPCR) were similar in all groups. All fetuses except one BDV-3 sheep were indeed positive for presence of BDV in at least one tissue sample. Mean viral loads were 4.7, 5.5, 5, 5.8 and 4, 3.4, 3.8, 4.6 log₁₀ RNA copies/100 mg of tissue for brain and thymus of BDV-3, BDV-4, BDV-5 and BDV-6 groups respectively. Differences (anova) were only significant between BDV-5 and BDV-4.

Conclusions: This study shows that strains of the four BDV genotypes harbor the same ability to infect pregnant ewes and pass through the placenta to infect fetuses. However, differences were observed between genotypes in their ability to induce fetal death and abortion, fetal malformation or growth retardation. These results must be taken into consideration when testing efficacy of vaccines to prevent production of permanent infected lambs. Further studies are warranted to assess the mechanisms of fetal pathogeny. The genome of each BDV strain was just fully sequenced and the comparative results will be discussed (data not shown).

SR2-021-006

Elimination of virulent strains (aprV2) of *D. nodosus* from feet of 28 Swiss sheep flocks – a proof of concept study

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Objectives: Ovine virulent footrot is a contagious foot disease and occurs in many countries worldwide. The disease is associated with severe pain, affecting animal welfare and causing economic losses. Achieving and keeping a high animal welfare standard and reducing antimicrobial use is of increasing societal relevance. Therefore, the Swiss government decided to study the option of implementing a nationwide footrot control program. The aim of this study was to evaluate the feasibility of elimination of virulent strains (aprV2) of *Dichelobacter nodosus* (*D. nodosus*) from sheep flocks by weekly footbaths.

Materials and Methods: The study was performed in 28 flocks (flock size minimum: 11; maximum: 115; median: 55) with a history of a footrot outbreak between April and December 2015. Initially every sheep was examined for clinical signs of footrot and scored according to the Swiss Health Service for Small Ruminants scoring system (score 0-5) adapted from Egerton and Roberts (1971). A four-feet-pooled cotton swab sample was taken of a maximum of five sheep with clinical signs in order to confirm the infestation with the virulent strain aprV2 of *D. nodosus*. Claws were carefully trimmed. Thereafter, a footbath of the entire flock during 10 minutes using a zinc sulphate (10%) disinfecting solution was performed weekly. Follow-up controls took place in three to four weeks intervals until a successful sanitation was achieved. At controls, every sheep was clinically scored, a four-feet-pooled sample taken and the claws were trimmed if necessary. The DNA was extracted according to

Stäuble et al. (2014). The PCR allowed distinction between the virulent protease aprV2 and the benign protease aprB2 of *D. nodosus*. The flocks were considered successfully sanitised if all sheep were aprV2-negative in the PCR. Follow-up evaluation of all sanitised flocks was performed between March and May 2015.

The correlation between the duration of sanitation and clinical prevalence or flock size, respectively, were analysed with the Spearman correlation in NCSS10. Risk factors for animals being aprV2-positive in the PCR were analysed with a logistic regression model in SAS 9.4.

Results: All 28 farms (100% of the study farms) were successfully sanitised. Seven farms were successfully sanitised after six weeks and the last ones (n=2) after 19 weeks. The number of footbaths (= duration in weeks) was highly correlated with the clinical within-flock prevalence at first visit and the mean score of the flock at first visit. No significant correlations were found between the flock size and the duration of the sanitation or the within flock prevalence at first visit, respectively. Sheep with high scores ≥ 3 were 28 times more likely to be aprV2-positive as compared to sheep with score 0. A number of > 6 footbaths was identified as protective factor.

Conclusions: A complete elimination of aprV2-positive sheep kept segregated from other sheep flocks and ruminants is possibly by weekly footbathing in zinc sulphate (10%), careful removal of loose claw horn and culling of non-responders, without broad scale use of vaccinations or (local or systemic) antibiotics. The duration of the sanitation depends on the initial clinical prevalence of footrot and the mean initial lesion severity score. Further research is warranted in order to optimise the described sanitation program, aiming at increasing farmers' compliance.

SR2-021-007

Estimating the prevalence of ovine *Sarcocystis* spp. from abattoir surveillance of adult slaughter sheep from Tasmania

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Objectives: The objectives of this study were firstly, to estimate the prevalence of *Sarcocystis* spp., and secondly, to examine for trends in estimated *Sarcocystis* spp. disease prevalence recorded during routine meat inspection of Tasmania adult slaughter sheep during the last quarter of 2007 and first two quarters of 2013. The aim was to use exploratory data analysis of routine abattoir surveillance data as a means of identifying whether additional producer extension efforts, disease control programmes or specific epidemiological studies examining this cystic parasitic disease in Tasmanian sheep flocks are required.

Materials and Methods: Routine abattoir surveillance data (unpublished) were provided from 1 September 2007 to 30 June 2013 and included date of inspection, local government authority (LGA), abattoir identity (n=9) and the State of inspection (Tasmania, Victoria or South Australia). The dataset provided total count data for the number of sheep killed and, number of sheep inspected, age and sex of each line, and number recorded with macroscopic lesions consistent with *Sarcocystis* spp. in each slaughter line (n = 1960). Data analyses were performed in Microsoft Excel 2007 (Microsoft) and Stata version 13.1 (Statacorp, Texas). Overall surveillance period *Sarcocystis* spp. prevalence (total n sheep recorded with gross *Sarcocystis* lesions divided by total n sheep inspected) and estimated annual *Sarcocystis* spp. prevalence were calculated, noting that 2007 and 2013 were incomplete datasets

for this period. The effect of categorical predictors of State, abattoir and LGA on the dependant variable; recorded line prevalence of *Sarcocystis* spp., were examined in separate fixed-effect linear regression models. Mixed-effects linear regression models were also used to examine the effect of abattoir identity on line prevalence when state was included as a random-effect and abattoir was nested within state. No significant interactions were found. Statistical significance was set as $p < 0.05$.

Results: The dataset represented 355,071 Tasmanian adult slaughter sheep of which 325,325 (91.6%) were inspected across nine abattoirs in three States. Over half of the population (60.0%) was inspected across two abattoirs in Tasmania ($n=195,084$) with 39.8% inspected across 6 abattoirs in Victoria ($n=129,576$) and 0.2% ($n=665$) inspected at a single abattoir in South Australia. Macroscopic lesions of *Sarcocystis* spp. were recorded in a total of 46,381 sheep, equating to an overall surveillance period prevalence of 14.3%. There was variation in the estimated annual disease prevalence, ranging from 0.02% in 2008 to 23.8% in 2011. Tabulation of data by abattoir identity also illustrated clear differences in between-abattoir prevalence of *Sarcocystis* spp. and findings were supported by regression modelling. Regression analysis identified that abattoir identity ($p < 0.001$) was a significant predictor of estimated *Sarcocystis* spp. within-line prevalence in both fixed- and mixed-effects models. Compared to the baseline abattoir for comparison, higher levels of *Sarcocystis* spp. were estimated in lines of sheep inspected at three abattoirs ($p < 0.001$). Inspected lines of sheep of known LGA were also recorded with lower levels of *Sarcocystis* spp. ($p < 0.001$) compared to those of unknown/unrecorded LGA.

Conclusions: Reasons for significant between-abattoir differences in the levels of *Sarcocystis* spp. recorded during routine meat inspection of Tasmanian adult slaughter sheep are currently unknown. This may represent sampling bias, with sub-sets of slaughter sheep going to different abattoirs of varying tiers with different market access, variation in the diagnostic sensitivity of meat inspection across different abattoirs, or differences in the level of registrations, line speeds and inspection and recording speed. Overall, these preliminary findings suggest that further investigation into differing *Sarcocystis* spp. prevalence estimates, is warranted.

Comments: We gratefully acknowledge Animal Health Australia (AHA) for providing the dataset and particularly Lorna Citer for her support. The project was initiated during a World Universities Network (WUN) research mobility exchange programme between the University of Bristol School of Veterinary Science and University of Sydney Faculty of Veterinary Science and was hosted by the Bruce Jackson and Rowena Bell of the Tasmanian Government Department of Agriculture.

SU-036-001

A new predilection site of *Mycoplasma bovis*: outbreaks of postsurgical infected seromas in beef cattle

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Objectives: In 2014-2015, several Belgian Blue (BB) beef farms in the northwestern region of Belgium reported non-responsive fever, chronic weight loss and seroma formation after caesarean section (CS) in their cows and heifers combined with high mortality. The objective of the present study was to describe involvement of *Mycoplasma bovis* in these outbreaks.

Materials and Methods: All animals included in this study were adult BB cows that recently underwent CS, originating from 3 different farms (farm 1-3) of the same veterinary practice. All animals were examined clinically and with ultrasound. Samples were taken of seroma's and other affected organs and plated on Columbia blood agar (CBA) plates supplemented with 5% sheep blood and on a modified pleuropneumonia-like organism (PPLO) agar plate (DIFCOTM, BD, NJ), incubated at 35°C in an atmosphere enriched with 5% CO₂. Incubation was 48h for CBA plates, and 7 days for PPLO. Bacterial isolates were subsequently identified to the species level with standard biochemical methods. Presumptive *M. bovis* identification was based on the typical fried-egg colony appearance on modified PPLO agar and the presence of lipase activity as tested on medium containing tween-80. After mycoplasma species confirmation by realtime PCR targeting the *uvrC* gene of filter-elongated isolates, strain typing was performed by insertion sequence (IS) elements profiling (Southern blotting) on 6 *M. bovis* isolates, from three cows of two farms.

Results: In farm 1, 4 affected cows could be sampled. Three cows arrived November 2014 (cow 1-3). They had been sick for 2-6 weeks and had been treated with various antibiotics. Two cows (cow 1&2) were euthanized for welfare reasons. These animals were emaciated, with presence of large abscesses (pus filled) and seromas (clear fluid filled) throughout the abdomen. They both had mastitis on four quarters and arthritis on multiple joints. Culture of joint and udder was positive for *M. bovis* in both cases. Abscess fluid of one cow was cultured and contained *M. bovis* and *Aspergillus fumigatus* (different abscesses). Cow 3 appeared normal on the first ultrasound, but did have a *M. bovis* mastitis. She developed two separate seroma's in the following weeks, that both contained pure culture of *M. bovis*. The seromas were drained, and after extensive treatment she could return to the farm. A fourth cow from the same farm arrived in April, with arthritis, mastitis and multiple seromas on ultrasound as well. Again *M. bovis* was isolated in pure culture out of an abscess. Two different herds (herd 2 and 3) reported the same pathologies in June, with again isolation of *M. bovis* from seromas.

IS elements typing showed an identical *M. bovis* strain in animals from herds 1 and 3 (cows 4 and 6, respectively). In herd 1 the isolates from cow 3 (November) and cow 4 (April) were very similar, especially when using ISMbov1 IS elements, but not identical. Within one animal the same strain could be isolated from the udder, joints and the seromas (cows 4 and 6).

Conclusions: In contrast to humans, to the author's knowledge involvement of *Mycoplasma* spp. in wound infections has not been reported in animals. Most likely *M. bovis* spread from a chronic site of infection (mastitis/arthritis) to the surgical wound, possibly aided by physiological immunosuppression in late gestation. The present report underscores the poor prognosis in these cases and the facility by which a single clone can cause similar problems in neighboring farms.

SU-036-002

Toe necrosis, risk factors and a practical surgical intervention to a save cow's life.

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Objectives: Toe necrosis (TN) is a very serious claw-horn affection of especially the tip of the claw whereby the protective horn capsule is no longer present and direct contact between the sensitive pedal bone and surrounding (floor) is possible and present. More than 25 cows were surgically intervened and the results were evaluated based on the presence or absence of lameness

Materials and Methods: Twenty-five cows were diagnosed by MH for the presence of TN and if present a decision to remove the intervention was discussed with the dairy farmer. Data about breed, parity and stage of lactation were collected and the affected claw (front vs hind and medial vs lateral) were registered. After application of ligature around the metacarpus or the metatarsus 10 ml lidocaine or a comparable alternative was injected and after some minutes the surgical removal was performed just behind the necrotic part of the bone. The wound was covered with tetracycline spray and a pressure bandage, which was changed after 4 days and 7 days and completely removed after day 10. Antibiotics were not applied parenterally, a block was placed at the other claw at the same leg

Results: All the 25 cows included in this study were reintroduced in the regular housing situation at 10 days after the intervention, while no bandage was present and the cows were still walking on a block beneath the other claw at the same leg. Very clear was the absence of any lameness almost directly after the removal of the necrotic part. Completely covering of the wound was seen 4 months after intervention (gap was closed), better production and fertility was estimated the size of the claw increased to an almost normal size

Conclusions: In the Netherlands very good experience with resection/removal of the tip of the claw (pedal bone) was gathered with the help of good local anaesthesia. It is simple, takes 10-20 min., the lameness disappears completely and the cow functions normal again (for years) after 10 days bandage. A very simple and practical solution for a very painful situation.

Comments: Nowadays we pay more attention to a good pain management to minimise the pain of the patient and to optimize normal food intake to guarantee better recovery with a minimal loss of milk production.

SU-036-003

Conservative and Surgical Treatment in 36 Ruminants with Limb FracturesAntonio Carlos L. Câmara ^{1,*}Larissa R. Andrade ¹Julio Rafael M. Pereira ¹Marcel B. D. Passos ¹Suyan B. Campos ¹José Renato J. Borges ¹¹Hospital Escola de Grandes Animais da Granja do Torto, Universidade de Brasília, Brasília, Brazil

Objectives: Fractures are encountered on a regular basis in large animal practice. Decision between treatment and euthanasia depends on the economic or genetic value of the animal, the cost of the treatment and the prognosis associated with the particular fracture. Treatment choices vary between stall rest, external coaptation, external fixation and open reduction with internal fixation, depending on the type of fracture and the bone involved. The aim of the present study was to report the main locations of limb fractures in 36 ruminants and to determine the efficiency of the treatment choices.

Materials and Methods: A retrospective study in ruminants with limb fractures attended in two Brazilian Veterinary Teaching Hospitals was performed. Fifteen sheep, twelve cattle and nine goats were included. Conservative treatment (CT) with full limb casts (FLC) or FLC with modified Thomas splint were the method of choice in ruminants with distal fractures and isolate cases of humerus, radio and tibia fractures. In cases of proximal or exposed distal fractures, the ruminants were treated surgically (external fixation, intramedullar pins or plats and screws). In three cases of exposed fractures with extensive bone necrosis and soft tissue damage, limb amputation was the treatment choice.

Results: The higher frequency of fractures involved the metacarpal or metatarsal (n=16; 44.4%) followed by fractures of the tibia (n=10; 27.8%), radius (n=5; 13.9%), femur (n=3; 8.3%) and isolated cases of medial phalanx and humerus fractures (2.8% each). Twenty one ruminants were treated conservatively and achieved a success rate of 95.4% (20/21). Surgical treatment was performed in 12 ruminants and achieved a recovery rate of 100%. Three ruminants were euthanized by owners request. Overall recovery rate reached 91.7% (33/36). Complications included dropped fetlock in the contralateral limb (2/36), osteomyelitis (2/36), cast sores (8/36) and muscle atrophy (36/36). Normal biomechanical limb movement and muscle strength was reestablished in six weeks at the most.

Conclusions: Our results confirms that ruminants are excellent orthopedic patients because of great bone healing properties, limb immobilization tolerance and rare contralateral limb disorders. Use of a Thomas splint and FLC combination is appropriate for fractures distal to the elbow or stifle as observed by our results proving its efficiency in reducing fractures of metacarpal, metatarsal, tibia, humerus, and radio in the treated ruminants. Internal or external skeletal fixation should be considered an option in the treatment of metacarpal, metatarsal, tibial and femoral fractures in ruminants, especially in low body weight animals.

SA-030-001

Technical Feasibility and Experimental Development of Sand Recycling Options for Dairy Cattle Bedding

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Objectives: Sand is considered the 'gold standard' product for dairy cow bedding in the UK, due to its comfort, absorbency and inorganic nature which inhibits pathogen growth. However, decreasing availability of sand and increasing financial pressure on farm is forcing other considerations such as Recycled Manure Solids (RMS) - more controversial with regards safety and pathogen growth.

Aims:

- 1) Characterise cattle bedding sand (physical, chemical, biological) before and after use
- 2) Compare characteristics of sand to other cattle bedding types
- 3) Review current literature on bedding use /recycling
- 4) Assess recycling efficiency of 6 cattle bedding treatments

Materials and Methods: This investigation was carried out using grab samples of pre-use and used sand bedding material from one farm in North Yorkshire with good practice and well managed sand bedding and pre-use Recycled Manure Solids (RMS) from another farm to characterise the material based on chemical (Carbon, Nitrogen, Phosphorous, Calorific value), physical (qualitative analysis of material structure) and biological characteristics (using baseline respiration, substrate induced respiration, and biogas production potential). Bedding samples were then treated at lab scale with processes which could potentially be implemented in the field i.e. dry heat treated (550°C), single autoclaved (125°C for 18 minutes), double autoclaved (using the principle of tyndallisation, 125°C for 18 minutes times two with 24 hours between treatments), or washed (replicating movement of water in a roller and brush type slurry separator), or with a combination of washing and single autoclaving (as outlined above). Following the processing of the samples, characterisation re-occurred with the exception of calorific value as the pre-use, used and RMS samples produced calorific values all below detectable limits. A complimentary basic pathogen analysis was also carried out on the pre-use, in-use, processed and RMS samples

Results: Pre-use and used sand were physically, chemically and biologically distinct. Sand structure was fine to medium grained, sub-rounded homogenous silicate of grain size of (100 – 500 µm). After use approximately 80% of the material was as pre-use, with the addition of organic matter collections of 1-5cm diameter. Significantly higher levels of moisture, organic content, respiration potential and pathogen counts were noted in post-use sand when compared to pre-use sand.

RMS (alternative bedding option), displayed distinct qualitative and quantitative qualities to pre-use and post-use sand. The structure of RMS was very different from sand, appearing as very low density, fibrous heterogeneous material, with fibres of up to 3cm in length. RMS chemical and biological characteristics (before use) (i.e. moisture content, organic content, respiration potential and pathogen counts), were far higher than either clean bedding sand or post-use bedding sand. RMS was exempt from treatment methods, as the standard treatment is to dry this material and reuse.

Post-treatment of sand bedding: no significant differences in the physical properties of the sand based on microscopy based inspection. Dry heat treatment of the sand at 550°C in an oven reduced respiration potential to below that of clean, pre-use sand and samples contained no mastitis causing pathogens after treatment. Washing used sand significantly lowered respiration potential and pathogen colonies relative to the

post-use levels. Autoclaving was deemed to be least successful as a treatment method, and enhanced biological activity.

Conclusions: For pathogen removal and returning sand to its original state of comfort and absorbency, autoclaving was ineffective.

Washing samples reduced sand to pathogen levels of pre-use sand; when combined with autoclaving, the number of mastitis causing pathogens counted increased: combining washing with autoclaving has no advantage.

The most effective treatment method was to dry heat treat the sand at 550°C. This returned sand to its original physical properties and completely removed mastitis causing pathogens from the sand.

Future work is required to optimise conditions for treatment options and evaluate cost benefit and societal acceptance.

SA-030-002

The growing importance of Farm Animal Para professionals (Vet Techs) as part of a Veterinary Led team in the UK.

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Objectives: The report on veterinary expertise in food animal production by Philip Lowe in 2009 argued that the veterinary profession needed to draw closer to its customers. It recommended that an area to be explored was 'the appropriate development of the roles of veterinary technicians'. Farmers are also increasingly under pressure to reduce costs including veterinary spend.

This presentation/poster outlines how a large animal practice in the South of England has grown this part of their business, the roles that can be performed, the role of the veterinary surgeon in managing the business and the potential for a new income stream.

Materials and Methods: A large veterinary practice in the South West of England servicing 45,000 dairy cows with 28 vets is described. The para professional team is described in detail and the varied roles that they perform throughout the business. The management structure and quality control and audit of these staff is discussed as well as HR considerations. The recruitment and continued professional development of these staff is discussed and the challenges of staff retention. The various financial models for such a service are discussed including the main one used in this business.

Results: 15 veterinary technicians (paraprofessionals) are employed by the practice. These include 7 qualified foot trimmers, 2 clinical veterinary technicians, 2 dairy hygienists, 3 SQPs (suitably qualified persons) and a laboratory technician.

Some of the services on offer include Cattle Foot trimming, mobility scoring, general animal handling, freeze branding, sampling, assisting vets with routine procedures such as debudding, vaccination, specialist fertility work (including ET), laboratory diagnostics, milking machine troubleshooting, client training, advice on worming strategies and trial work.

Conclusions: The provision of this service to the farm animal sector is vital to the ongoing supply of veterinary services on farm. The authors believe that retaining these skills under a veterinary led team allow a joined up approach on farm while facilitating quality data collection as well as maintaining animal health and welfare. The authors highlight that they see the Veterinary Technician/Para professional as potential the future for the farm animal veterinary sector.

SA-030-003

Impact Of Routine Hormone Treatments For The Reproductive Management Of Dairy Herds On Methane Emissions And Reproductive Efficiency

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Objectives: The aim of this study was to evaluate the magnitude and between herd variation of changes in methane emissions and economic efficiency associated with 3 simple hormonal interventions to manage breeding in early lactation.

Materials and Methods: Data for 10,000 herds of 200 cows were simulated. Probability of conception was predicted daily from the start of the study (at a calving) for each cow, up to day 300 of lactation. Four scenarios of differing first insemination management were simulated for each herd, using the same theoretical cows: A baseline scenario based on breeding from observed oestrous events only, synchronisation of oestrous for fixed time first insemination using 2 methods, and a regime using prostaglandin treatments followed by first insemination to observed oestrous events. Cows that did not conceive to first insemination were re-inseminated following detection of oestrous. For cows that conceived, gestation length was 280 days with cessation of milking 60 days before calving. Those cows not pregnant after 300 days of lactation were culled and replaced by a heifer. Daily milk yield was calculated for 730 days from the start of the study for each cow. Change in mean reproductive and economic outputs were summarised for each herd following each intervention. For each scenario, methane emissions were determined by daily forage dry matter intake, forage quality, and cow replacement risk. Linear regression was used to summarise relationships between input and output variables.

Results: In some circumstances improvement in reproductive efficiency using the programmes investigated was associated with reduced cost and methane emissions compared to reliance on detection of oestrus alone. Efficiency of oestrus detection and the time to commencement of breeding after calving influenced variability of changes in cost and methane emissions. For an average UK herd there was a saving of at least £50 per cow and a 3.6% reduction in methane emissions per L of milk when timing of first insemination was pre-set. The global warming potential of this change is equivalent to that from 2 cars, a family home, or 21 barrels of oil.

Conclusions: Use of hormonal interventions in the reproductive management of dairy herds can be controversial. In some circumstances for every litre of milk produced, both economic and environmental benefits through reduced methane emissions occurred. However, this was not universal across all farm scenarios simulated. It is therefore important that hormonal interventions can be prioritised; for circumstances associated with a demonstrable benefit to society in terms of food security and sustainability, in addition to economic efficiency for the farmer.

SA-030-004

Preliminary results of an investigation of methods of establishing deep cubicle beds of recycled manure solids for dairy cows in the UK

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Objectives: Deep beds of recycled manure solids (RMS) can provide comfortable beds for cows. However, farmers have reported that the bedding may not dry out optimally, and heating may occur, if a large amount is initially put into cubicles. Such conditions could influence microbial growth and adversely affect udder health. It has been suggested that building up the beds gradually may be preferable. This experiment was designed to test whether building beds gradually was associated with drier bedding and less heating. In addition the impact of the presence of cows during the bed building phase was assessed.

Materials and Methods: The study was conducted at Sewborwens Farm, Newton Rigg, Penrith, Cumbria, CA11 0AG, UK. Sixteen cubicles were used for the trial, four being allocated to each of four treatments:

- 1) Rapid Fill with Cows (RC): Beds filled to capacity with RMS on day 1 with cows having access.
- 2) Rapid Fill no Cows (RNC): Beds filled to capacity with RMS on day one with no cows having access.
- 3) Slow Fill with Cows (SC): Beds filled with shallow layers of RMS daily, aiming to reach full capacity on Day 7 with cows having access.
- 4) Slow Fill no Cows (SNC): Beds filled with shallow layers of RMS daily, aiming to reach full capacity on Day 7 with no cows having access.

The trial was replicated twice in two different sets of cubicles in the same shed.

On days one to seven, the depth of bedding and the temperature at 2.5 cm depth were recorded in each cubicle, and a sample (approximately 100g) of bedding was collected from the top 2.5 cm of the bed for determination of dry matter (DM). On day 29, the total depth of bedding was recorded and temperatures were measured and samples were collected for DM analysis at 2.5 cm depth intervals. Temperature, relative humidity and wind speed in the shed were recorded by data loggers throughout the study.

Data from the two replicates were analysed separately, to compare bed parameters between the four treatments, using non-parametric tests in view of the wide variation evident in the data.

Results: Replicate 1 demonstrated that bedding in rapid filled cubicles with cows reached the highest temperatures during the week of establishment (17 oC vs 11-12 oC for slow filled). DM content was lowest in slow filled with cows (36% v 38-39%, $p < 0.05$). After four weeks the slow built beds were significantly cooler at 5 cm depth and tended to have a higher DM content at Day 29 than rapidly filled beds, but the effect of occupation by cows was no longer significant. In Replicate 2, under warmer environmental conditions, both rapid fill treatments reached higher median temperatures (19-24 oC) than slow fill treatments (14 oC) ($p < 0.05$) during week one, but the presence of cows did not have a significant influence during this time. In contrast to Replicate 1, DM % was higher with slow filling than with rapid filling during the building phase (39-42% v 36-38%, $p < 0.05$). After four weeks the bed temperature with cows present was higher than without, but the only significant difference between individual treatments was a lower temperature in SNC compared with other treatments ($p < 0.05$).

Conclusions: Rapid building of beds elevated temperatures of bedding in the building phase, and the effect appeared to persist for four weeks. Temperature was affected more by speed of fill than by the presence of cows. The effect of building speed on DM content at the surface during the first week and four weeks later was inconsistent. Dry matter content of the surface material appears to be influenced by factors in addition to

the speed of bed building which may include the presence of cows. There appears to be a complex interaction between environmental conditions, including temperature and relative humidity, and the temperature and DM of RMS bedding.

Comments: This has been submitted under Udder Health as it will be related to the paper submitted by Andrew Bradley et al, but you may decide it is more appropriate in another section?

SA-030-005

Carbon footprint of sheep production systems in the Mediterranean climate zone of Chile: Simulation model.

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Objectives: A simulation model developed previously was modified with the aim of estimating the carbon footprint of extensive sheep production systems in the Mediterranean climate zone of Chile, with the purpose of determining the influence of operating variables and the pattern of rainfall on carbon footprint and productivity. Together, the relative importance of ecosystem service "carbon sequestration" (grassland and soil) on the carbon footprint of sheep extensive systems was quantified.

Materials and Methods: Main pastoral resource for sheep production in the region correspond to the natural prairie basically the called Mediterranean annual pasture. Other grazing species observed in the area correspond to subterranean clover, falaris and oats. On the other hand, the use of cereal stubble is a necessary and common practice during the summer months. The sale of the lambs, in these systems is usually performed during the months of September to December, with animals from three to four months of age and weighing between 28-35 kg., generally unweaned. To assess the carbon footprint Cradle to farm-gate methodology proposed by FAO (2010) was used, which implies that emissions of greenhouse gases were assessed for all processes involved until that the lambs leave the farm. The production of carbon per kilogram of live weight of lamb was the functional unit used to assess the carbon footprint. The estimation of GHG emissions based on the equations of Tier 2 proposed by IPCC (2006) was realized. Three typical farms present in the zone were simulated. Three pattern of precipitation (dry, normal and wet) were used for to determinate the grassland growth. Three supplementation levels, five levels of stocking rate variation and grassland with and without fertilization were simulated. Factorial experiments were used to evaluate the effect of changes in stocking rate, supplementation level of sheep and fertilization of natural pastures (and weaning) about the carbon footprint (CO₂-eq/kg live weight of lamb). The simulations were realized with and without the valuation of ecosystem services.

Results: All factors studied had a significant effect on the carbon footprint, weight of lambs, weight of sheep and grassland availability. Interactions of second, third and fourth orders were observed. Average carbon footprint obtained was 14.8 CO₂-eq/kgLW. The incorporation of carbon sequestration did decrease this value to 2 CO₂-eq/kgLW. Increasing stocking rate caused an increase in the carbon footprint, but this trend was reversed with the addition of carbon sequestration. The weights of lambs and ewes, as well as the final grassland availability decreased to the extent that increased stocking rate. The effect of supplementation

during the period studied was the smallest on the studied variables. Fertilization grassland (and weaning) elevated the carbon footprint due to the decrease in the weight of lambs and emissions from fertilizers used, but increased the weight of ewes. The years with lower rainfall showed the highest carbon footprints and caused significant decreases in the values of the other variables studied.

Conclusions: The model allows predicting the effect of management changes and precipitation pattern on the carbon footprint and productive variables. Incorporating some of the ecosystem services provided by sheep production systems modifies the effect of the studied factors on carbon footprint. An integral assessing of sheep production systems for assessing their impact on the production of greenhouse gases is necessary. The model developed is a first approximation.

TP1-045-001

Comparison of Two Macrolides in the Control of BRD in Calf Ranch CattleNorman Stewart ^{1,*}¹Technical Services, Merck Animal Health, De Soto, United States

Objectives: The purpose of this study was to determine the health performance of calf ranch cattle at high risk for developing bovine respiratory disease (BRD) after the administration of two different macrolides for the control of respiratory disease.

Materials and Methods: Holstein heifers (165 - 240 lbs). predicted to be at high-risk for BRD were utilized in this trial. BRD incidence, with no previous use of an injectable BRD control agent, was 20%.

On the same day, all animals were individually weighed, their temperature taken, BVD PI tested, randomly allocated antibiotic given after they left their hutch at +/- 60 d of age and prior to entering their randomly assigned pen.

Treatment groups were Tildipirosin: 4 mg/kg (1.0 ml/cwt), s.c., 1 x with a 3 day post metaphylactic interval (PMI) or Tulathromycin: 2.5 mg/kg (1.1 ml/cwt), s.c., 1 x with a 3 day PMI.

Animals were placed in equal sized pens (max 55/pen, 2 paired pens per replicate, 22 replicates on test). 1,030 animals received Tildipirosin and 1,029 received Tulathromycin at enrollment. Workers then observed the animals daily for clinical BRD signs. They were blinded to treatment and trained to uniformly score clinical signs. BVD PI animals were removed from the study.

When an animal was sick after 3 day PMI, 40 mg/kg florfenicol, 2.2 mg/kg flunixin meglumine, 6 cc/ cwt., s.c., 1 x with a 4 day post treatment interval (PTI) was injected. Clinical signs included nasal and ocular discharges, respiratory rate and character, presence and character of cough, demeanor (depression), and gauntness. Based on any one of these clinical signs, animals were treated for BRD. Animals with one or more signs were assigned a depression and respiratory score (0=normal, 1=mild, 2=moderate, 3=severe).

All animals were weighed off test the same day, +/- 42 d on test,

Results: There was a statistical difference ($p=0.032$) with regards to morbidity following the administration of the respective treatment groups' antibiotic for the control of BRD. Ninety-five animals or 8.46% (95/1,030) of the animals treated with Tildipirosin broke with BRD compared to 128 animals or 11.43% (128/1,029) of the animals treated with Tulathromycin.

There were no statistically significant differences ($p < 0.05$) between the two treatment groups with regards to respiratory and depression scores, treatment success rates following treatment with 40 mg/kg florfenicol: 2.2 mg/kg flunixin meglumine, mortality, chronics, relapses and body temperatures.

Conclusions: This large field study demonstrated that treatment with Tildipirosin was superior to Tulathromycin in reducing Bovine Respiratory Disease morbidity with both antibiotics having a 3 day PMI, when used to control BRD in calves.

TP1-045-002

Efficacy of flunixin meglumine pour-on administration in a tissue cage model of inflammationOlivier Roy ¹René Fournier ²Julien Thiry ²Philippe Brianceau ³Siddhartha Torres ^{3,*}¹CEBIPHAR, Fondettes, ²MSD Santé Animale, Beaucouzé, France, ³Merck Animal Health, Madison, United States

Objectives: The objective of this study was to evaluate in cattle the effect of flunixin meglumine applied topically in reducing PGE2 concentration in exudates after induction of subcutis inflammation using a tissue cage model.

Materials and Methods: Six healthy not-lactating dairy cows (514 ± 42 kg) and six healthy grass calves (369 ± 49 kg) were included in the study and randomly assigned to two treatment sequences. Sterile hollow perforated polyethylene balls were surgically embedded in the subcutis at four distinct sites per animal three weeks prior to D0. On D0 and D14, an aseptic inflammation of the subcutis was induced by injecting in three balls per animal 0.5 ml of a 2% carrageenan solution. Carrageenan challenge was immediately followed by the topical treatment on the dorsal midline area: NaCl or flunixin meglumine (3.33 mg flunixin meglumine / kg body weight). Exudate was collected prior to challenge and 2, 4, 8, 12, 24, 36, and 48 hours after. Cages were emptied after each collection. PGE2 concentrations were measured in exudate using a validated liquid chromatography coupled with mass spectrometry method. Each animal received the two items as treatment sequentially on D0 and D14 according to group assignment.

Results: In NaCl-treated animals, PGE2 concentration levels displayed a sharp increase, peaked 8 hours after challenge, and gradually decreased over time (PGE2 concentration levels were still elevated 48 hours after challenge). In flunixin meglumine treated animals, PGE2 peak occurred later (12 hours after challenge) and was strongly reduced compared to that in NaCl treated animals: PGE2 concentrations were consistently lower than those measured after NaCl administration. Percent of inhibition was close to or over 90% at the peaks of PGE2 concentrations (8 and 12 hours after challenge) after flunixin meglumine treatment, and inhibition lasted until the end of the animal phase. The log ratio of concentrations for the two treatment groups are significantly different (linear mixed model, p values ≤ 0.05) at hours 8, 12, 24, 36 and 48, but are not significantly different at hours 2 and 4.

Conclusions: This study shows that flunixin meglumine applied topically inhibits carrageenan-induced subcutis inflammation in cattle. Anti-inflammatory effects occurred as soon as 2 hours and lasted at least for 48 hours post-administration.

TP1-045-003

Early detection of bovine respiratory disease and immediate treatment with flunixin help to reduce the use of antibiotics in beef calvesC. C. Gelfert ^{1,*}C. Ströbel ²C. Beisl ³E. Thesing ⁴D. Lange ¹¹Intervet Deutschland GMBH, Unterschleissheim, ²Ströbel Christina_Beratungsdienst Rindermast, Ilshofen, ³Gassner Franz & Beisl Josef_Tierärztl. Gemeinschaftspraxis, Frontenhausen, ⁴Intervet Deutschland GMBH, Unterschleissheim, Germany

Objectives: The use of antibiotics (AB) is monitored in Germany since midyear 2014 and beef farmers are forced to implement new strategies to reduce the use of AB. New tools to detect fever in calves provide the opportunity to detect bovine respiratory disease (BRD) in an early stage and to treat these animals with an NSAID alone. In the early stage of BRD, viruses are the main pathogen, thus an antibiotic may not be necessary. Then, treatment with an NSAID alone may be sufficient to both inhibit inflammation and control the infection.

The hypothesis of our study was that an early treatment of BRD in calves with flunixin will reduce the use of AB by 33%.

Materials and Methods: The study was performed on 2 beef farms in southern Germany. New arrived calves (n= 40 per farm) were clinically examined and vaccinated intranasally against BRSV and PI3V. A probe (fevertag®) was installed in one ear to measure constantly the calves body temperature. A fever tag alarms by a red flushing light if body temperature is constantly > 39.7°C for 6 h. Fever alarms were monitored for 8 weeks. During this time each fever alarm was reported and allocated randomly to two treatment groups. A repeated alarm on the same calf was counted as a new case if the time interval between two alarms exceeded 7 days. On both farms antibiotics were fed orally for the 1st 14 days, a general method in Germany for new arrived calves on beef farms.

In a case of an alarm (=d 1), rectal body temperature confirmed fever (>39.7°C). In case of fever they were randomly assigned to 2 treatment groups and treated the same day. Calves assigned to the control treatment (n=37) received one injection of florfenicol and flunixin. Calves assigned to the study treatment (n=43) were treated once with flunixin as pour on (3.3mg/kg). All calves were clinically examined on d 2. If fever or clinical signs of BRD were observed further treatment was performed with florfenicol. Calves were finally examined for clinical disease and fever on d 6 to close a case.

Descriptive parameters were used to describe the effects of the different treatments. Frequency of additional treatment in the study group (and 95% CI) was calculated. The sign-test was performed to test our hypothesis.

Results: During the study period, 80 cases of fever alarm were monitored, 24 calves showed two fever periods. Mean body temperature at first treatment was 40.2°C. Besides a slight increased lacrimation in a few calves, no other clinical signs were visible. Appetite was not reduced in all but two calves.

Of the 80 cases, 37 cases were treated with florfenicol and flunixin and all but three calves did not require further treatment. 43 cases were allocated to the study treatment and treated with flunixin only. In 29 cases (67.4%, 95% CI [50.5%; 80.9%]) no further treatment till day 6 was necessary. According to the sign test, the reduction in the use of AB was significantly above the 33% of our hypothesis (P=0.0315).

Conclusions: In case of early BRD detection, these results show that early treatment with flunixin reduced the use of AB in fattening calves by 67%. This seems to be an effective strategy for beef farmers to comply with the new German legislation to reduce antibiotic usage. Further studies should show whether these tools of early detection also help to minimize metaphylactic treatments with antibiotics in beef cattle farming.

TP1-045-004

Chemical residues in dairy cattle following use of organic therapies for mastitis

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Objectives: Many consumers in the United States are demanding that food animals be raised without using antibiotics as evidenced by the increase in certified organic food production. There is great incentive to assess alternatives to antibiotics. Many plant essential oils, extracted from plant material, have been historically used as antibacterial and anti-inflammatory agents. This presentation will focus on the pharmacokinetics of two chemicals in many antibacterial plant essential oils, thymol and carvacrol, that are major components of several products used on organic dairy farms in the U.S.

Materials and Methods: An intramammary (IMM) product (Phyto-Mast, CowMaster LLC, Narvon, PA) containing both carvacrol and thymol or a topical udder product (Uddersol, Ralco Animal Health, Marshall, MN) which contained only carvacrol was administered at 1X or 2X the recommended dose to healthy mid-lactation Jersey cross dairy cows (n = 10) in a randomized design. Plasma and milk samples were collected periodically for up to 5 days after the last dose. Tissue samples were also collected to assess residue depletion in liver, kidney, muscle and fat. All samples were analyzed by headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME-GC/MS/MS). All milk, plasma, and tissue analytical methods were developed and validated in our laboratory. The limit of quantification (LOQ) was 0.0005 µg/ml for all matrices. Pharmacokinetic noncompartmental modeling was performed using Phoenix® (Certara, St. Louis, MO, USA) to estimate plasma and milk pharmacokinetic parameters for each chemical.

Results: IMM administration of Phyto-Mast resulted in thymol and carvacrol early peak plasma concentrations (< 0.02 ppm average) at about 25 minutes that declined rapidly to below limit of quantification (LOQ) within 12 hours. The apparent plasma half-life of thymol and carvacrol were 1.69 hr and 1.70 hrs, respectively. Peak milk concentrations were at 4 hrs for most cattle and ranged from 0.5 – 9.0 ppm thymol and 0.5 – 12 ppm carvacrol, but declined to below LOQ within 12 hours.

Topical Uddersol resulted in peak plasma carvacrol concentrations (< 0.002 ppm average) at 1-4 hours with levels below LOQ after 12 hours. Comparisons of plasma area under the curve (AUC) values suggested a 2-fold greater relative bioavailability of carvacrol from Phyto-Mast than from Uddersol. As anticipated, carvacrol concentrations in milk were also greater with Phyto-Mast than Uddersol as peak carvacrol levels from the latter were always less than 0.35 ppm.

Preliminary tissue concentrations for thymol and carvacrol from IMM Phyto-Mast were relatively low in liver (0.0068 ppm and 0.0051 ppm), kidney (0.010 ppm and 0.0066 ppm) and muscle (0.0058 ppm and 0.0066 ppm), respectively. Topical Uddersol resulted in low levels of carvacrol in kidneys (0.0118 ppm), fat (0.0072 ppm), muscle (0.0062 ppm), and liver (0.0027 ppm). All tissue concentrations were at the reported peaks 24 hours after slaughter and had dropped below the LOQ by 96 hours.

Conclusions: We demonstrated that chemical components in IMM or topical mastitis therapies can be detected in milk for several hours and in tissues for just a few days after the last dose. Thymol and carvacrol were cleared from the milk within 24 hrs but persisted longer in various organs and edible tissues. These estimates are based on a limited dataset

and further analyses are required. With the growth of the organic dairy industry in the United States and global concerns over antibiotic use in livestock, it is important to evaluate these and similar alternatives to antibiotics to ensure food safety.

TP1-045-005

Sodium iodide inactivates *Manheimia hemolytica* and *Bibersteinia trehalosi* in vitro.

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Objectives: Bovine respiratory disease is an important cause of economic loss in the cattle industry. The airway epithelium has several innate defense mechanisms to protect the health of bovine lungs; one of these is lactoperoxidases (LPO) and hydrogen peroxide (H₂O₂) which react with excreted halide ions to form hypohalous acids on the epithelial surface. The objective of this study was to determine if two important bovine bacterial respiratory pathogens, *Manheimia hemolytica* and *Bibersteinia trehalosi* (formerly *M. hemolytica* biotype T), are susceptible to inactivation by the LPO/H₂O₂/halide system that is present in bovine airways.

Materials and Methods: Clinical isolates from bovine pneumonia cases presented to the diagnostic lab for post-mortem examination were used in this study. Isolates were identified via traditional biochemical testing and confirmed via Maldi-tof. Experiments were carried out on three different strains of each bacteria. The susceptibility of these pathogens to inactivation by hypiodous acid was determined by generating HOI in vitro using LPO, sodium iodide (NaI) and H₂O₂. Concentrations of NaI from 50 µM to 500 µM were tested, and controls were run in parallel. Subsamples were removed prior to addition of H₂O₂ (catalyst) and at 5 and 30 minutes. Bacterial killing was assessed via standard plate counts. Experiments on each strain were performed in duplicate, on three separate occasions.

Results: Results of previous studies have showed this system to be effective at inactivating human respiratory syncytial virus and adenovirus. In this study we found that the complete reaction mixture (LPO, H₂O₂, NaI) killed both of our bacterial pathogens, *M. hemolytica* and *B. trehalosi*, at all concentrations of NaI tested (50-500µM). Bacteria was killed by 5 minutes after initiation of the reaction. A surprising finding was that NaI alone at higher concentrations (above 250µM) inactivated both pathogens immediately in vitro.

Conclusions: NaI and its product, hypiodous acid, show exciting promise as a preventative strategy for bovine respiratory disease. In human studies, orally administered NaI results in elevated levels of iodide in respiratory secretions. Introducing high levels of iodide in airway secretions in cattle at times of peak stress and susceptibility to respiratory disease may prove a valuable preventative strategy for respiratory disease, which may also help to reduce antimicrobial use in food producing animals.

TP1-045-006

Prevention of Neonatal umbilical infections in Holstein calves through accelerated desiccation of the umbilical remnant utilizing a novel therapy

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Objectives: Reduction of navel infections through appropriate management including navel dipping is beneficial to the calf and the producer and is reported to decrease navel infection rates from 20-28% to 5-14%. Obviously, navel dipping is of great importance but obtaining tincture of iodine has become problematic. It has become necessary to develop other products that can be utilized to dry the umbilicus and assist in the prevention of navel infections. Hence, the objective of this study was to evaluate an alternative to 7% tincture of iodine, Super7+™ Navel Dip.

Materials and Methods: A total of a 100 neonatal Holstein heifers were utilized in this study. Fifty calves were dipped with treatment A, (Super7+™ Navel Dip) immediately following calving and 50 were dipped with treatment B, (7% tincture of iodine). The umbilicus and the umbilical remnant of all calves were evaluated 48 hours following dipping and at least a 1 cm segment of umbilical remnant was removed and placed in a labeled airtight container. All samples were analyzed within 6 hours of sampling and in 12 hour increments until the samples contained less than 10% moisture. A serum sample was collected from each calf within 48 hours of birth and tested for total protein, immunoglobulins (IgG) and specific gravity. The workers dipping the calves and the technicians analyzing the samples were blinded to the treatment.

Results: Of the remnants undergoing treatment A, 88% and 100% were desiccated within 48 hours and 60 hours, respectively. Of the umbilical remnants undergoing treatment B, 58% and 100% were desiccated by 48 hours and 60 hours, respectively. There was a strong association between treatment A and drying at 48 hours. (P = 0.0008, Mantel-Haenzel Chi-Square). The odds of drying out at 48 hours were 5.31 times higher with treatment A compared to treatment B. There was no clinical evidence of umbilical infection in any of the calves utilized in this study. The total protein, IgG and specific gravity were analyzed from all calves (mixed linear models) and were determined to not be significantly different between treatments (P = 0.415, P = 0.439, and P = 0.300, respectively).

Conclusions: Treatment A (Super7+™ Navel Dip) appears to be superior to tincture of iodine in its ability to more quickly desiccate the umbilical remnant. Hence, Treatment A (Super7+™ Navel Dip) appears to function competently as a navel dip and is a viable alternative to 7% tincture of iodine.

TP2-046-001

Treatment of bovine respiratory disease in juvenile calves with a single administration of a combination of florfenicol plus flunixin meglumine

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Objectives: Whether Bovine Respiratory Disease (BRD) causing factor is physical, environmental, or infectious, a sequence of events occurs resulting in inflammation and ultimately activation of the innate and adaptive immune systems. It is advisable that NSAID and anti-infective agents are used concurrently to treat BRD. The present study intended to confirm the efficacy and the safety of the florfenicol-flunixin combination formulation for the treatment of BRD in juvenile calves less than 6 weeks of age in comparison to an approved positive control, florfenicol, that represented a negative control in regards to flunixin meglumine.

Materials and Methods: A total of 210 calves of less than 6 weeks of age, originating from France, Germany and Spain, and showing severe signs of respiratory disease (depression score ≥ 2 , respiratory score ≥ 2 and rectal temperature $\geq 40.3^{\circ}\text{C}$), were enrolled and randomly assigned to treatment. Each animal was randomly assigned to treatment with either the test product, 300 mg/ml florfenicol plus 16.5 mg/ml flunixin formulation (Resflor®; 40mg/kg florfenicol and 2.2mg/kg flunixin; 2ml/15kg; MSD Animal Health), or the control product, 300 mg/ml florfenicol formulation (Nufloor®; 40mg/kg; 2ml/15kg; MSD Animal Health). Both products were administered once subcutaneously in the neck on day 0. To avoid observation bias and to preserve masking, treatments were dispensed and administered by individuals not involved in the clinical assessment. All animals were observed daily for depression, respiratory character and rectal temperature for 10 days post-treatment. From day 4 to day 10, animals with a depression score ≥ 1 or a respiratory score ≥ 2 that had a rectal temperature $\geq 40.0^{\circ}\text{C}$ were classified as treatment failures and removed from the study. The dosing sites was evaluated and any adverse effects were recorded. All animals underwent nasopharyngeal swabbing for bacterial culture, identification and determination of florfenicol sensitivity.

Results: The predominant respiratory pathogens were *Pasteurella multocida*, *Mycoplasma bovis*, *Mannheimia haemolytica* and *Histophilus somni*. All isolates were found susceptible to florfenicol.

In both groups, rectal temperature significantly dropped and clinical index (depression and respiratory signs) significantly improved after treatment. Specifically, for the change in rectal temperature from pre-treatment to 6 hours post-treatment, the florfenicol-flunixin formulation was found significantly superior to florfenicol ($p < 0.0001$). Moreover, the florfenicol-flunixin formulation alleviated the clinical signs of disease more rapidly, and was demonstrated non-inferior to florfenicol on days 4 and 10 success rates.

Neither florfenicol-flunixin formulation nor florfenicol had a negative influence on the health status including appetite and faecal consistency, confirming that both products are safe.

Conclusions: Prompt treatment of sick calves with a NSAID (e.g. flunixin) in conjunction with a broad-spectrum antibiotic (e.g. florfenicol) helps reduce fever, control bacterial replication, and reduce the likelihood of lung consolidation. This study demonstrates that the single administration of Resflor® can be used successfully to treat juvenile veal calves with respiratory disease.

TP2-046-002

Evaluation Of An Alternative Therapy For Infectious Bovine Keratoconjunctivitis

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Objectives: Infectious bovine keratoconjunctivitis (IBK), commonly called "pink eye", is a painful condition affecting beef and dairy cattle worldwide. The bacterium, *Moraxella bovis* is known to be responsible for this condition. Antibiotic therapy has been primarily utilized for this condition. However, there is a need to reduce the amount of antibiotics utilized in food animals. Hence, the objective of this research was to evaluate an alternative therapy (Vetericyn Plus™ Pinkeye Spray) to promote healing and aid in the reduction of pain and infection due to experimentally induced IBK.

Materials and Methods: Thirty dairy bull calves having determined to have normal ophthalmic examinations and who were culture negative for *M. bovis* were randomly assigned to three groups for a single eye block randomized blinded challenge study. Calves were housed in pairs according to their respective group in an approved isolation facility. On day zero a 0.6 mm corneal lesion was made on the center of the left corneas of Groups 1 and 2 utilizing n-heptanol. Immediately following lesion formation, 1.0×10^7 of *Moraxella bovis* (strain Epp63-300; origin: NADC) was administered topically to the left central corneas of Groups 1 and 2. The calves in Group 3 (Control group) received topical corneal administration of *M. bovis* to their left eyes but nothing further. Starting on day one, each calf in Group 1 received two mLs of Vetericyn Plus™ Pinkeye Spray topically to each cornea twice daily for 10 days. Additionally, calves in Group 2 each received two mLs of 0.9% Saline topically to each calves' cornea twice daily for 10 days. Each animal was given a pain score twice daily (based on blepharospasm, ocular discharge and tearing). All eyes were cultured on day -7, 0, 1-5, and day 10. Digital images were taken of each eye of each calf from day 0 to day 10 of the study. The lesions were measured daily utilizing Image-J technology. Additionally, serum and plasma samples were drawn from all calves on days 0, 1, 10, 11, and 17 and evaluated for changes in sodium and chloride levels. Statistical evaluation was performed utilizing SAS® and a Kenwood-Roger correction was utilized.

Results: All calves in group 1 and 2 developed lesions in the left eye as determined by fluorescein staining. All calves in group 2 developed lesions consistent with IBK in the left eyes. Calves in group 2 only were determined to be culture positive for *M. bovis* during the study period. Between Days 1 and 2, Group 1 had significantly, $P < 0.05$, decreased pain scores when compared to controls. On average there was a reduction in pain score by 79.1% by day 2 and an 83.7% reduction in pain by day 10 when compared to controls. Group 2 had an average reduction in pain score of 18.3%, and 67.9% by day 2 and by day 10, respectively, when compared to controls. There was no significant difference in sodium and chloride levels in the plasma and serum among all three groups at any of the sampling time points ($P < 0.05$). The days to cure was significantly different between Group 1 and Group 2 ($P = 0.0161$) and there was a significant difference in the treatments when evaluating lesion width and circumference ($P = 0.0147$) and ($P = 0.0375$), respectively.

Conclusions: The results of this study indicates that an alternative therapy (Vetericyn Plus™ Pinkeye Spray) can significantly aid in the reduction of pain, infection and healing time of corneal lesions in calves experimentally infected with *M. bovis*.

TP2-046-003

Altered Plasma Pharmacokinetics of Ceftiofur Hydrochloride in Cows Affected with Severe Clinical Mastitis

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Objectives: The objective of this study was to compare the plasma pharmacokinetics of ceftiofur hydrochloride between healthy dairy cattle and those afflicted with severe clinical mastitis. Our hypothesis is that cows affected with severe infectious disease will have altered ceftiofur pharmacokinetics relative to healthy cows, necessitating variance in dose regimens and withdrawal periods

Materials and Methods: Eight cows with naturally occurring mastitis and eight clinically healthy cows were treated with 2.2 mg of ceftiofur hydrochloride/kg of body weight once daily for five days via the intramuscular route. Blood was collected at 0, 0.33, 0.67, 1, 1.5, 2, 3, 4, 8, 16, and 24 hours after the first ceftiofur administration and every eight hours thereafter until 120 hours after the final dose. Plasma samples were analyzed for ceftiofur concentrations using liquid chromatography coupled with mass spectrometry. Ceftiofur concentrations are reported as total ceftiofur which includes the parent compound and all metabolites. Single dose and multi-dose non-compartmental pharmacokinetic models were determined for both groups.

Results: With the exception of time 0, ceftiofur was detected at all time points. The disease group had a significantly higher plasma ceftiofur concentration at T=3 h after the first injection and a significantly lower plasma concentration from 40-152 h following the first injection, with the exception of the T=64 h time point. Data following the first injection (Time 0-24 h) were fit to a single dose, non-compartmental pharmacokinetic model. This model indicated that the disease group had a shorter plasma half-life. A multi-dose, non-compartmental model was used to determine steady state pharmacokinetics. Compared to control cows; the disease group had an initially higher peak concentration, a higher volume of distribution and drug clearance rates. The disease group also had a lower area under the curve per dosing interval, steady state concentration maximum, and dose-adjusted peak steady state concentration. All other pharmacokinetic parameters were not different between the two groups.

Conclusions: These data show that significant pharmacokinetic changes occur in diseased animals administered ceftiofur relative to healthy, control animals. These outcomes potentially have public health significance in that: 1) drug efficacy could be lower than expected; 2) there may be an increase in violative drug residues in tissues of cull animals or milk; and 3) this may lead to increases in antimicrobial resistance. Further work examining protein binding, tissue distribution, and tissue depletion of drugs and their influence on residue levels in diseased animals is necessary to more thoroughly characterize this problem.

TP2-046-004

Use of Antibiotics on United States Dairy Operations

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Objectives: Veterinarians are the primary health providers and/or consultants on most U.S. dairy operations. In these roles, veterinarians are usually involved in the selection and use of antibiotics. Preventing drug residues in milk and meat has always been a priority for veterinarians, and guidelines for judicious use of antibiotics are available to dairy practitioners. The use of antibiotics in livestock agriculture is coming under increased scrutiny because of the focus on reducing antibiotic resistance. The objective of this study was to describe the use of antibiotics on dairy operations.

Materials and Methods: The National Animal Health Monitoring System's (NAHMS) Dairy 2014 study was conducted in the nation's top 17 dairy states. These states represented approximately 80% of U.S. dairy operations and 80% of U.S. dairy cows. During March through July 2014, veterinary medical officers or animal health technicians visited 265 dairy operations that had at least 30 cows. Multiple questions from the study's questionnaire focused on antibiotic use during 2013 in the following cattle classes: preweaned, weaned, pregnant, and adult. Statistical software, which accounted for the complex study design, was used to provide estimates reflective of the U.S. population of dairy producers.

Results: Ionophores were fed to weaned heifers on 50.5% of operations and to 62.7% of all weaned heifers. Similarly, 39.3% of operations, representing 46.8% of all pregnant heifers, fed ionophores to pregnant heifers. Cows were fed ionophores on 37.0% of all operations. Treatment with intramammary antibiotics at dryoff was practiced on 88.5% of operations, and overall 93.0% of all cows were dry treated. For dry-cow therapy, third generation cephalosporins were used on 22.3% of dry-treated cows. Diarrhea was the most common disease in preweaned heifers: 21.3% of all heifers were affected with diarrhea, and 15.9% of were treated with antibiotics for diarrhea. The primary antibiotics given for diarrhea were third-generation cephalosporins (28.1% of treated preweaned heifers), trimethoprim/sulfa (19.2%), and aminoglycosides (14.9%). Respiratory disease affected 8.2% of weaned heifers. The primary antibiotics used for respiratory disease were florfenicol (32.2% of treated weaned heifers) and macrolides (29.3%). The percentage of cows treated with antibiotics for common diseases were 20.8% for mastitis, 7.8% for reproductive disease, 3.7% for lameness, 2.7% for respiratory disease and 1.2% for digestive disease. On operations that treated cows with antibiotics, third-generation cephalosporins were used to treat cows for mastitis on 50.8% of operations, lameness on 59.6%, respiratory disease on 78.3% and digestive problems on 61.9% of operations.

Conclusions: Ionophores are frequently used as feed additives on dairy operations, but this class of antibiotics is not listed as 'medically important' by the FDA and, thus, is not affected by the recent FDA guidance documents. Third-generation cephalosporins, trimethoprim/sulfa, and macrolides are all considered critically important for use in humans. However, these drugs are being used in the dairy industry primarily for disease treatment purposes, and relatively few cattle are treated with these drugs.

TP2-046-005

Comparison of tildipirosin and tulathromycin for control of bovine respiratory disease in high-risk beef heifersRiccardo Compiani ¹Stefano Jottini ²Siddhartha Torres ^{3,*}¹Practitioner, ²MSD Animal Health, Milano, Italy, ³Merck Animal Health, Madison, United States

Objectives: The Italian beef system is based on fattening young calves imported from abroad. Cattle are inevitably subject to stressful transport conditions, which last on average 8 to 12 hours. Even under excellent management conditions and administration of well-designed immunization protocols, preventive antimicrobial treatment is often required to reduce morbidity and mortality due to Bovine Respiratory Disease (BRD). The purpose of this study was to evaluate the effectiveness of tildipirosin (TIP) and tulathromycin (TUL) administered to newly received beef heifers at risk to develop BRD for reducing morbidity and mortality under field conditions.

Materials and Methods: A total of 785 Charolais heifers considered to be at high risk of developing undifferentiated BRD were enrolled in the study. Heifers were grouped and randomly allocated in pair to receive either tildipirosin (ZUPREVO® TIP; n=398; at 4 mg/kg) or tulathromycin (DRAXXIN® TUL; n=387; at 2.5 mg/kg). Morbidity due to BRD was defined as animals showing mild to severe depression and/or respiratory signs and having a rectal temperature $\geq 104^{\circ}\text{F}$ (40°C). Animals diagnosed with BRD (first episode) were administered 2 doses of florfenicol at 9.07 mg/ lb (20 mg/ kg) 48 hours apart. Average daily gain (ADG) was also recorded. Data collected at slaughter included cold-carcass weight, dressing percentage, and lung lesions within a subgroup of animals (n = 433; 207 for TIP vs 226 for TUL). Continuous variables were analyzed with Student's t-test. Variables expressed on either a binary or ordinal scale were analyzed using the chi-square test.

Results: BRD morbidity was lower in the TIP group compared to heifers in the TUL group (6.8% TIP vs 20.9% TUL; $P < 0.01$). At the end of the 125-day feeding period, heifers in the TIP group were heavier ($P < 0.01$) and had 0.15 lb (0.07 kg) greater ADG ($P < 0.01$). Carcasses of TIP group animals tended to be heavier at slaughter ($P = 0.06$), but there was no difference in dressing percentage. Nearly 70.7% of first-pulled animals did not have lung lesions at slaughter, therefore, metaphylaxis supplemented by effective therapeutic treatment was an effective strategy under the conditions of this study.

Conclusions: Under the conditions of this study of high-risk heifers, treatment at arrival with tildipirosin reduced morbidity compared to treatment with tulathromycin. Lower BRD morbidity favorably influenced ADG. Health history before arrival at the feedyard is an essential element of information for accurate BRD risk assessment of beef cattle

TU1-017-001

Development and Evaluation of an Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection of Bovine Tuberculosis

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Objectives: Cell-mediated immune (CMI) responses are the principal and earliest immune responses to develop after infection with *Mycobacterium bovis*, the causative agent of bovine tuberculosis. In contrast, antibody responses develop considerably later than CMI responses but from a practical perspective serological based test assays are useful tools to complement the diagnostic portfolio for bTb testing because of the stability of antibodies during sample transport, storage and handling. Here the development of an ELISA for the detection of bovine tuberculosis is described – the PrioCHECK™ Tuberculosis Complex Ab

Materials and Methods: The PrioCHECK™ Tuberculosis Complex Ab is an indirect ELISA test to detect antibodies directed against *Mycobacterium bovis* antigen which is coated in wells of a microtiter plate. Serum samples are incubated in the wells of the plate and *Mycobacterium bovis* specific antibodies are captured by the immobilized antigen on the plate followed by detection with a peroxidase (POD) labeled anti-bovine IgG. The PrioCHECK™ Tuberculosis Complex Ab can be used for single sample analysis as well as for high throughput testing applications.

Results: A total of 835 samples were tested with the prototype PrioCHECK™ Tuberculosis Complex Ab (541 positive and 294 negative samples). 50 samples derived from an Irish and 204 samples from UK negative herds, defined on historical records of > 5 years bTb free history based on skin test and BOVIGAM™ negative test results. Furthermore 200 negative samples from the USA and 56 negative samples from Switzerland were tested. 11 samples provided by the APHA were confirmed as bTb positive reactors (skin test and BOVIGAM™ positive test results). In addition 38 positive Irish, 100 positive US and another set of 138 UK positive samples were analysed. The ELISA cut-off was set at 15% positivity based on receiver operating characteristic (ROC) analysis. The measured diagnostic sensitivity was about 66 % (95 % CI, 60.3 - 71.4), the specificity about 98 % (95 % CI, 96.2 – 99.2).

Cross-reactivity was determined with samples from *M. kansasii*, *M. avium* and *M. avium* paratuberculosis. 46 *M. avium* paratuberculosis positive samples from Ireland, the Netherlands and the USA were measured. In addition 8 *M. kansasii* and 8 *M. avium* positive samples from the USA were determined. Overall, none of the 62 samples analysed showed reactivity in the ELISA.

Conclusions: The PrioCHECK™ Tuberculosis Complex Ab is a suitable and reliable tool for the detection of bovine tuberculosis. It shows no cross-reactivity towards other mycobacteria. The determined sensitivity of 66 % and specificity of 98 % makes it an ideal tool to complement the diagnostic portfolio for the detection of bovine tuberculosis.

TU1-017-002

The effect of seasonality on ranging behaviour in Irish badgers.

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Objectives: The ranging behaviour of badgers and their movements through the environment are of direct importance to the transmission of tuberculosis (TB) infection both between individual badgers and their social groups, and also between badgers and the cattle on the farms through which they travel. The objective of this study was to investigate the year-round ranging behaviour patterns of male and female European badgers in a medium density population in Co. Wicklow, Ireland. The effect of sex and age of the badgers and seasonality, in terms of temperature, rainfall and photoperiod, was also evaluated.

Materials and Methods: Over a three-year period, from May 2010 to May 2013, 19 male and 19 female badgers were fitted with Global Positioning System (GPS) tracking collars (Tellus Light collar by Followit, Sweden). The collars were programmed to take either four or eight GPS readings per night according to the season. When eight GPS readings or attempted readings had been recorded in the collar's memory, the collar sent a text message to Followit in Sweden, who then relayed the positional data to the authors by e-mail. The resulting data was converted to Irish grid and used to calculate home range estimates. Data analysis was conducted using the statistical environment "R" (version 2.14.1). Home range estimation was done using the minimum convex polygon (MCP) method and the local convex hull (LoCoH) incorporating the 'adaptive sphere-of-influence' version (a-LoCoH). Generalised linear mixed models (GLMMs) were used to describe changes in ranging behaviour. Factors such as sex, age, month, temperature, and rainfall were examined and all possible models were generated and ranked using Akaike Information Criteria for small samples (AICc scores).

Results: 33928 GPS readings were received from the 38 badgers. The maximum home range recorded for a male was 724 hectares (ha) in April 2013 and 323 ha for a female in June 2010. The home ranges varied greatly in size between individuals (e.g. between 576 ha and 35 ha in the same month). The mean home range size for all males was largest in April 2013 at 287 ha, while the mean for females was largest in June 2010 at 166 ha. A seasonal cycle in range size was clear with summer ranges being significantly larger than winter ranges. Male and female badgers had similar range sizes up to their second year. Males aged 3-5 years had the largest home ranges. Males reduced their winter ranging less than the females and the females entered winter lethargy more than the males did.

Conclusions: This study showed that male badgers over three years had the largest home ranges, whereas females and younger badgers of both sexes had smaller ranges. Males were flexible regarding winter lethargy and maintained a full home range through the winter in some years. These results are important for understanding the dynamics of TB transmission both within badger populations and between badgers and cattle. In addition, our findings may aid the optimisation of vaccine delivery programmes by allowing the targeting of specific age and sex cohorts.

TU1-017-003

Temporal association between badger presence and herd bovine tuberculosis (bTB) risk in County Monaghan, Ireland

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Objectives: In Ireland, badgers are culled in response to herd breakdowns of bovine tuberculosis (bTB), where implicated following an epidemiological investigation of the 'index herd' episode. Our goal was to quantify relative temporal trends in bTB risk before and after culling of badgers from the land of surrounding herds (those with 33% of land area within 500m of an affected sett) in county Monaghan during 2004-2011.

Materials and Methods: To describe herd 'exposure' in relation to badger presence we assumed that a culled badger had been present around the sett for the previous 6 months (exposure period). After badger removal, we assumed that surrounding herds would experience a period of decreased badger presence ('post-exposure period'). We further assumed that this decreased badger presence might affect the outcome of a herds next bTB test, provided that appropriate time-lags were observed (eg to account for test latency).

The first Cox survival Models (Model A) tested the null hypothesis that there was no decline in herd bTB hazards in the 'post-removal' period (i.e. the next herd test ≤ 15 months later). Model B, tested the hypothesis of no difference in bTB hazard before badger removal compared with all other times. The outcome was a herd experiencing a bTB episode. In both A & B, we controlled for herd size, enterprise type, count of previous bTB episodes, being within a former experimental removal area, and being an index herd.

In A, we estimated the earliest date when the next herd test could be counted as 'post-badger removal'. We accounted for possible test latency by including a range of time-lags. (ie with 'badger removal date' delayed by 0, 1, 2 and 3 months). Models were compared using Akaiques' Information Criteria (AIC); the lowest AIC lag model was considered "best". The resulting time-lag was used to define the end of the exposure period for B.

Results: Over the period 2004-2011, 860 setts were approved for capture in relation to 112 index herds. A total of 1,102 badgers were captured at 354 of these setts on 748 occasions. In relation to the 860 setts, 1,155 surrounding herds were selected as the study population (25% of herds in the county). Following sett approval, 178 bTB episodes occurred in 159 of the surrounding herds. 140 herds experienced 1 and 19 experienced 2 bTB episodes.

Model A

A negative relationship between herd bTB risk and increasing months to the next test was observed in all four versions of Model A, as measured by the time-varying covariate for the competing models of timelags 0, 1, 2 and 3 months. A time-lag of 2 months (AIC = 2,287) was selected as the lowest value, (i.e. the model with most plausible time-lag). In Model A, decreasing hazards were observed from 2 months after badger removal until the next test (equivalent to a 17% decrease in hazard per month; $p=0.003$).

Model B

Prior to badger removal, the hazard ratio was significantly increased (H.R. = 1.82; $p=0.001$), compared to other times.

Conclusions: The finding of decreasing hazards for bovine TB in cattle following badger removal, and increased hazards for herd bTB during the period of assumed badger presence adds to the already compelling evidence of an association between bovine TB in the two species. Since the study population represents only a subset of herds within 1 county in Ireland, caution should be applied in generalising these results more widely.

TU1-017-004

Does spill-over of bovine tuberculosis from badgers occur at cattle yards or troughs, or by direct contact ?

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Objectives: Spill-over of bovine tuberculosis from badgers to cattle is important, but where and how this spill over occurs, remains unclear. Spotting such spill-over events might be achieved if we knew more about this, they may be by badgers visiting cattle yards or troughs, or by direct contact at pasture. It is a subject of some controversy, in an already controversial, and difficult topic. Over recent decades, badgers have been detected in cattle yards, and at troughs, in Ireland both north and south, and in southern England. The objective here is to review data generated in Ireland recently, and compare that with findings from England.

Materials and Methods: Methods used in Ireland to study this included field observations, rainfall recording to see if it influenced results, sand bands, cameras, radio-tracking, proximity collars, GSM collars and in addition, winter surveys of 200, 100 beef and 100 dairy, randomly selected yards. The latter surveys were for badger signs, especially tracks in mud, at a time of year when cattle were indoors, in County Cork. Work in Northern Ireland was in County Down. Both areas had TB blackspots. In England there was more emphasis on selected farms, as well as marking badgers, radio-tracking, video surveillance, and rainfall recording.

Results: Very few signs of badgers were detected in yards, and it appears that badger tend to avoid them in Ireland. The accuracy of the ability to find badger tracks in winter was tested using two teams one of which realised badgers and one of which checked for badger tracks the next day. Badgers have also been detected troughs in both parts of Ireland. In England there was more emphasis on selected farms, as well as marking badgers, radio-tracking, video surveillance, and rainfall recording. One hundred and thirty nine visits by twenty six badgers were reported in one study in England.

Conclusions: Despite use of modern techniques spill-over is not yet understood. Considerable variation by season and month, herd/group size and climate variables as well as at an individual level were recorded in Northern Ireland. The ecology of both badger and cattle Ireland and Britain a different. This may explain why the results between the two countries, are conflicting, and difficult to compare.

TU2-018-001

Replacing reactive culling of badgers by badger vaccination: part of the end-game of the eradication of bovine tuberculosis in the Republic of Ireland

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Objectives: The objective of this work is to ascertain if intra-muscular (IM) vaccination of badgers, with BCG, is not inferior to area-wide targeted badger culling in maintaining a reduced herd-level risk of bovine tuberculosis (bTB) in the Republic of Ireland (ROI). Badgers (*Meles meles*) are an important reservoir of *M. bovis* in ROI. The current bTB eradication program involves an annual test/slaughter program in cattle plus localized targeted badger culls where epidemiological investigations link badgers to bTB breakdowns. The cull area in ROI covers circa 28% of agricultural land with circa 6,500 badgers removed annually.

Materials and Methods: Six counties in ROI were selected for the study. Suitable areas (at least 150 km² with road, water, or mountains as boundaries) within each county were deemed vaccination areas; the remaining area within the counties receive continued culling of badgers. Vaccination/release of badgers starting in County Longford in 2011. The project was rolled out in stages with all sites becoming fully operational by 2014. Testing data from cattle in these six counties will be analysed/evaluated. Our primary outcome of interest are herd breakdowns with 3 or more standard SICCT reactors during an episode (an episode is the interval a herd is prohibited from trading). Badgers in the areas designated for vaccination with BCG, will be captured, marked, vaccinated and released. Outside the designated areas in the participating counties badgers will be culled as per the national program. In the study counties, it is unlikely that vaccination will be superior to continued culling hence our interest is whether or not vaccination will lead to a bTB risk, in cattle herds, that is not inferior to that produced by the current culling program. Vaccination need just be not more than a defined amount worse (called the inferiority margin and represented here by Δ), than continued culling in terms of bTB risk in cattle herds. We denote the risk of this in herds in the current (or culling) program area as p_c and in the area with badger vaccination is denoted as p_v . Our null hypothesis is a one-sided comparison $H_0: p_v - p_c \geq \Delta > 0$ where $p_v - p_c$ is a small value that has little practical importance.

Results: Formal analysis of the results from the 6 counties sites will not take place until the end of 2017 and will cover the period 2014-2017. Results from one county, Longford, will be presented for the period 2011-2014. The outcome variable of primary interest is the proportion bTB breakdowns in herds resulting in the identification of 3 or more standard interpretation reactors. In county Longford, since vaccination commenced in 2011, larger breakdowns have remained below 1% of episodes overall and the risk of larger breakdowns has not been any higher in the vaccination zones compared to the rest of the county. Overall, nationally, there has been a circa 50% reduction in episodes of bTB in cattle herds since 2001 and these data will be presented in terms of the categories of breakdowns as defined by the numbers of standard reactors identified per episode in the study counties. Because badgers are a protected species, culling in the ROI is carried out under licences issued by the Heritage Dept. Targeted culling programs are undertaken that maintaining badger populations at reduced densities in areas where bTB is endemic in cattle and by association in badgers also. The current

capturing program commenced in 2003 and the area under capture has grown since then and now covers a cumulative area that is circa 28% of all agricultural land. How these culling areas evolved in the 6 study counties will be outlined, as will the trends in bTB herd breakdowns over the interval up to 2014.

Conclusions: This trial will end in Dec 2017 when a decision on whether using BCG vaccination in endemic TB areas in the ROI will be taken then. Vaccinating badgers, if not inferior to continued culling and therefore a suitable part-substitute for the continued culling of badgers which is currently carried out annually, will result in fewer badgers culled. A strategy that includes vaccination of badgers as a part-substitute for culling will be sustainable in the longer term as lower cull rates will not threaten the longer-term viability of ROI badgers. Such a strategy will contribute to the national goal of achieving TB-Free status by 2030.

TU2-018-002

The impact of local weather on European badger (*Meles meles*) capture success: implications for bovine tuberculosis management

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Objectives: Bovine tuberculosis (bTB) affects livestock and wildlife around the world. Badgers (*Meles meles*) are an important wildlife reservoir in Ireland and Britain, which complicates disease eradication efforts. In the Republic of Ireland, badgers have been managed through culling to control bTB, but vaccination using the intramuscular *Bacillus Calmette-Guérin* (BCG) vaccine is becoming the new goal. However, for vaccination to be effective, badger trapping success must be high to deliver vaccines to a large proportion of badgers in a given population (coverage). In this study, we examined the effect of local weather on badger capture success.

Materials and Methods: We compiled data from 1,985 badger captures during 2010-2013 as part of a bovine TB badger vaccine trial in County Kilkenny, Ireland. Trapping using stopped restraints and cage traps occurred at active setts (dens) for a period of 8 nights, with traps checked daily. At the start of a trap session, field staff assigned an activity score to the setts, based on a scale of 0-5. For each trap night, field staff collected data on minimum and maximum air temperature and assigned a precipitation score from 1-6, ranging from dry to snow. We compared Poisson, zero-inflated Poisson, negative binomial, and zero-inflated negative binomial models to evaluate factors affecting badgers capture numbers.

Results: In our preliminary analysis, we found that badger captures were significantly higher in drizzle (25%, $p = 0.01$), rain (22%, $p = 0.02$), and heavy rain (32%, $p = 0.006$), and significantly lower in snow (72%, $p = 0.03$) compared to dry weather. Badger captures were significantly higher (69%, $p < 0.001$) at setts with activity scores of 4 as compared to setts with activity scores of 3. There was a quadratic relationship between temperature and badger captures. We are currently continuing to analyze additional data to account for variation trapping effort (traps laid per sett).

Conclusions: Our results provide valuable information for prioritizing trapping based on local weather conditions. Increasing capture success will increase vaccination rates, which will increase protection against the development of bovine TB amongst badgers. Effectively vaccinating badgers will be a crucial aspect and a key step toward the goal of eradication of bovine TB in Ireland.

TU2-018-003

The role of tuberculin assays on naturally infected cattle in the Irish bovine tuberculosis eradication programme.

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Objectives: Periodic assays in naturally infected tuberculous cattle, as recommended by the WHO Technical report series 384, fulfils a critical function as part of overall quality control of the Irish bovine tuberculosis eradication programme in order to assure optimal diagnostic surveillance. This poster gives an account of the procedures and outlines the role of tuberculin PPD assays carried out on naturally infected cattle in the quality control on the Irish Bovine Eradication Programme.

Materials and Methods: Cattle are injected at 4 sites on either side of the neck. Injections are rotated sequentially through each of the eight possible neck sites in each of 24 cattle using the Latin square design so as to minimise any statistical bias due to variations in skin sensitivities at the different skin sites. The injection sites are clipped and the skin-fold thickness at each injection site is measured using a manual calliper with 1 mm graduations at 0 hours. The tuberculin PPDs are injected intradermally using McLintock syringes Skin fold thickness are re-measured at each site at 72 hours.

Results: The results are analysed using standard statistical methods for parallel-line assays according to Finney (1), using the GLM procedure within the statistical software package, SAS(SAS Institute Inc., 2003). The outcome measure is the increase in skinfold thickness after 72 hours and all models included a term for animal to account for repeated measures on the same animal. The concentration term is transformed using logs and included in the model as a linear term. Separate slopes are assessed for each tuberculin by testing the interaction term (log_concentration x tuberculin) within the model. This is the test for parallel slopes. If this interaction term is not significant then the relative potency can be calculated as the difference in average skin increase between the test and standard tuberculin divided by the slope (the increase in mean reaction per unit increase in log dose). Site and Side of the injection are included in the final model if significant. The 95% confidence limits for the relative potency are calculated according to Fieller's method (Finney, 1978)

Conclusions: Successive cattle assays have validated the results of guinea pig assays as batches found to have potencies of 30,000 [66-150%] I.U. per ml by guinea pig assay also exceeded this potency when assayed in cattle. Cattle assays are considered essential to ensure that EU requirements for tuberculin potencies are achieved and periodic assays in naturally infected tuberculous cattle remains necessary as part of overall quality control of the Irish bovine tuberculosis eradication programme.

TU2-018-004

Where and when are badgers (*Meles meles*) bovine tuberculosis positive? Data from culled populations across the Republic of Ireland

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Objectives: European badgers (*Meles meles*) are an important wildlife host of bovine tuberculosis (bTB; *Mycobacterium bovis*), and have been implicated in the epidemiology of bTB in cattle herds in Ireland and the United Kingdom (UK). The objectives of the study were to determine the spatial and temporal trends of bTB in badgers in Ireland, while also investigating associations between the characteristics of badgers and local cattle TB prevalence.

Materials and Methods: The bTB status of 4900 badgers culled (2009-2012) as part of a targeted policy of reducing badger density in areas where cattle herds experienced bTB "breakdowns" was determined using well established *M. bovis* culture techniques. We applied multivariable Generalised Estimating Equation (GEE) modelling and Geographic Information System (GIS) analysis to assess the spatial and temporal trends in badger apparent prevalence using these data. Risk factors assessed included badger characteristics (age class, gender, parity), sett (burrow) characteristics (size, distance to other setts), and local area cattle bTB prevalence.

Results: We found significant variation in badger prevalence in both space and time. There was a trend in apparent prevalence towards westerly regions having higher risk than easterly regions of the country. Over time there was a significant linear reduction in bTB risk. There was a clustering effect within the dataset, with animals caught within the same sett having a higher bTB status correlation than between setts. Furthermore, badgers caught at setts that were closer to other "infected" setts were at higher risk of testing positive for bTB. Badger infection risk was associated with local area cattle herd prevalence (within 1km of the badger sett) during the year when the badger was captured. Non-parous female badgers were found to have lower risk than parous-female or male badgers.

Conclusions: Over the study period the apparent prevalence of bTB in badgers in culled areas decreased significantly. However, there was significant geographic variation in prevalence across Ireland. We found a significant association between badger risk and local area cattle prevalence (≤ 1 km of the badger sett). This observational study adds evidence to the association between badgers and cattle concerning the epidemiology of bTB in Ireland. However, our data cannot determine the direction of infection. At an animal level, we found that the pregnancy status of female badgers significantly impacted on their likelihood of being culture positive for bTB.

TU2-018-005

The avoidance of farmyards by European badgers *Meles meles* in a medium density population.

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Objectives: The objective of this study was to see if badgers used farmyards as often as would be expected from their availability within the landscape. It also investigated the influences of farmyard type, season, gender and social group membership on the likelihood of a badger entering a farmyard.

Materials and Methods: This Irish study is the first to have looked at the use of a variety of farmyard types by badgers. Over a three-year period 40 free-ranging badgers from a medium-density population in Co. Wicklow were fitted with GPS collars. Locational data received from the collars were used to create home ranges for each badger in each season. A Farm Bias Index was developed using paired T tests with a Holm correction. GLMMs were used to examine the effects of the season, gender and social group variables.

Results: We found that badgers in our study area avoided all types of farmyards. This avoidance was significant in nine of the 12 seasons. Badgers particularly avoided those farmyards where cattle were present. If badgers did enter a farmyard it was more likely to be an equestrian yard or a disused farmyard than a cattle yard.

We found no impact of seasonality, social group membership or badger gender on this behaviour. Half the badgers in the study visited at least one yard over the three years. There was some evidence of individual preferences on the part of the badgers.

Conclusions: While our results differ from the findings of studies carried out in high-density badger populations in Great Britain, it is not possible to distinguish those density effects from differences in farming practices in GB. It is likely that badger behaviour and ecology as well as cattle stocking density and biosecurity must be taken into account when designing measures to control the transmission of this disease. The results of this study strengthen the growing body of evidence that transmission of TB between badgers and cattle is less likely to be through direct contact than through fomites or other indirect means.

Comments: *Mycobacterium bovis* (TB) in cattle is a zoonotic disease with far-reaching effects throughout Europe but especially in Great Britain and on the island of Ireland. Wildlife reservoirs, in particular the European badger *Meles meles*, continue to play an important role in the transmission of the disease, although the pathways of transmission are still poorly understood. The badger is an opportunistic feeder that takes advantage of seasonally abundant foods, such as fruits and soil invertebrates. Badgers might therefore be expected to enter farmyards to exploit grain stores or feed concentrates. This would bring them into close proximity with livestock present in those yards, potentially increasing the opportunity of disease transmission. Increasing our knowledge of the interactions between badgers and cattle in a variety of ecological situations is essential to assist in proactive and general control of TB in both species.

TU2-018-006

A preliminary assessment of oral vaccine bait uptake by wild badgers in Ireland

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Objectives: Culling remains a controversial method of maintaining low badger populations in areas where wildlife reservoirs of *Mycobacterium bovis* persist. Current long-term plans aim to replace culling with oral and/or parenteral vaccination of badgers. We investigated the uptake of vaccine-free baits by wild badgers across Ireland to assess suitable levels of bait density deployment and pre-feeding (prior to live vaccine bait deployment) for Irish populations.

Materials and Methods: We adopted and modified a bait deployment strategy developed by researchers in the UK. Bespoke baits were presented at setts targeted for control over a six-day period prior to trapping. Six baits were presented at sett entrances on each day using the following protocol:

Day 1 – naked baits (biomarker 1)
Day 2 – naked baits (biomarker 1)
Day 3 – packaged baits (biomarker 1)
Day 4 – packaged baits (biomarker 1)
Day 5 – packaged baits (biomarker 2)
Day 6 – packaged baits (biomarker 2)

By changing the biomarker in baits deployed later in the trial we simulated a “live” vaccine bait deployment on days 5 and 6.

Results: The results section cannot be completed yet, since we are still collecting data. Our baits will be deployed through the winter. Once we have collated the results, I anticipate the section to be formatted in a similar manner to the outline below.

We presented bait at ## setts across Ireland between ## 2015 and ## 2016. ## % of trapped badgers had biomarker 1 present in blood serum (X1 out of Y1 animals). ## % of trapped badgers had biomarker 2 present in blood serum (X2 out of Y2 animals).

Comparative levels of the two biomarkers indicated that ## % of trapped badgers ate pre-baits prior to our simulated “live” vaccine bait deployment.

Conclusions: This section cannot be properly formatted as we do not have any results yet.

We aim to answer these questions:

Q. How many baits per day are best for Irish setts?
A. Are 6 baits per sett per day enough?

Q. How many days of pre-baiting are needed prior to live vaccine deployment?
A. How many of the badgers were found to have biomarker 2 in their blood serum?

Comments: While it is not possible to provide results at this point, we are confident that we will have all the data collected and analysed in time for the conference.

TU2-018-007

Bovine tuberculosis in cattle and red deer in Southern Bavaria, Germany – can network analysis explain the routes of transmission?

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Objectives: From 2000 onwards, increasing numbers of bovine tuberculosis outbreaks were reported in Southern Bavaria, Germany. The disease was observed in cattle and wild-living red deer with results of DNA fingerprinting indicating a transmission both within and across host species. The objective of our study was to describe the spread and transmission of the disease amongst cattle using network analysis and overlaying the geographical information of cattle grazing areas with locations of TB-positive hunted deer. Additionally we wanted to gain further knowledge on the epidemiological relevance of alpine pastures in the current outbreak.

Materials and Methods: Data on TBcases in cattle and movement data of cattle originating from holdings localized in the Bavarian districts bordering the Alps were obtained from the national database for cattle and swine (HITDatenbank) and covered the study period from 2010 to 2014. Data on bTBcases in red deer were collected within the framework of the European study EMIDAERANet and of the regional follow-up study, the Bavarian TBMonitoring in red deer. Two networks were visualized: An egocentric contact network consisting of the contacts of TBpositive cattle and a trade network representing cattle movements between holdings that had been visited by positive cattle. Regression analysis was used to identify risk factors for cattle holdings.

Results: The contacts and movements of bTBpositive cattle could account for a noticeable amount of positive bTB results in cattle holdings, but not for all. Therefore, factors additional to the contacts and movements of cattle tested positive for bTB must have contributed to the spread of bTB in the alpine region of Bavaria. The calculated centrality measures reflecting the exposition (indegree, eigenvector centrality) and the potential of holdings to spread infection (outdegree, betweenness centrality) both seemed to correlate with the infection status of cattle holdings. These correlations could be used to identify cattle holdings potentially at risk of infection in riskbased surveillance or during future outbreaks. Alpine pastures visited by bTB-positive cattle and *M. caprae*-cases in red deer overlapped to a large extent in the region "Allgäu" suggesting inter-species transmission in this area. All applied methods identified alpine pastures to be crucial for the spread of bTB in the alpine Bavarian districts.

Conclusions: The combination of spatial description, network and regression analyses could provide valuable insights into the epidemiology of bovine tuberculosis in the alpine region of Bavaria and indicated the importance of summer pastures for disease spread.

UH1-011-001

Use of infrared thermography to diagnose mastitis in pre-partum dairy heifers

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Objectives: Heifer intramammary infection (IMI) in early lactation impacts negatively on milk production and longevity in the herd, and is associated with a higher risk of subclinical and clinical mastitis. The origin of heifer IMI remains uncertain, in part due to the challenge of establishing when infection occurs, making the targeting of preventative methods difficult. Generally, it requires use of invasive techniques to collect mammary secretion pre-partum, which can themselves increase the risk of infection. Therefore, the aim of this study is to evaluate the usefulness of thermography as an early detection system for abnormal udder health.

Materials and Methods: The study was conducted on one commercial dairy farm of 700 lactating animals. One hundred twenty Holstein-Friesian heifers in the last trimester of pregnancy were enrolled in this field study. All heifers were submitted twice to infrared thermography (IRT); the first and second sets of thermograms were taken approximately two months and two weeks prior to the estimated calving date, respectively. A FLIR E300 infrared camera (FLIR Systems Inc., Wilsonville, USA) was used for all the IRT measurements. Each set of thermograms included two projections of the udder, one caudocranial and one ventrodorsal, from a distance of 0.70m. Ambient temperature, shed humidity and rectal temperature were measured and recorded. IRT images were analysed using ThermoCAM Research 2.10 Pro (FLIR Systems, Danderyd, Sweden). Different analysis tools (polygons, lines and spot meters) were applied to obtain a set of descriptive parameters, including 'min', 'max', 'max-min' and average. At calving, individual quarter samples were collected aseptically for milk culture using standard aerobic bacteriology and for measurement of somatic cell count (SCC) by means of fossomatic. In addition, clinical mastitis data and DHI-measurements of SCC over the first 100 days of lactation were recorded. Statistical analysis has been performed using SPSS version 17.0 (SPSS Inc., Chicago, USA).

Results: The synchronization of IRT image collection with farm routines allowed an easy, fast and non-stressful procedure. From the initial one hundred and twenty heifers, six were lost to follow up (two before 50DIM, four were sold before 100DIM). A Pearson product-moment correlation coefficient was computed to assess the relationship between the 'max' temperature of back quarters areas in caudocranial and ventrodorsal projections. Overall, there was a strong positive correlation between both projections at first IRT (left $r=0.70$, $P<0.0001$; right $r=0.63$, $P<0.0001$) and second IRT (left $r=0.62$, $P<0.0001$; right $r=0.54$, $P<0.0001$). At calving 37% ($n=161$) of the 440 quarters were culture-negative with geometric mean SCC of 188,000 cells/ml, 33% ($n=147$) presented <10 colonies with geometric mean SCC of 187,000 cells/ml, 25% ($n=109$) presented IMI (≥ 10 colonies) and geometric mean SCC of 421,000 cells/ml; and 5% were contaminated ($n=23$; ≥ 3 different morphotypes). SCC was higher for quarters with IMI than for culture-negative quarters or those with <10 colonies (Students' t -test; $P<0.001$ and $P<0.01$, respectively). Negative correlation was found between 'max' temperature at first IRT and SCC of IMI quarters ($r=-0.74$, $P<0.0001$).

Conclusions: Preliminary results of the study indicate that a ventrodorsal projection of the udder can be used to obtain a representative IRT image of the quarters, which may be useful in the most common handling facilities available in heifer rearing units, cattle race or crush. Thermography 2 months pre-partum may give earlier indications of IMI than SCC at first milking. Further analysis of IRT descriptive parameters, SCC and microbiology data will provide additional information on the capacity of IRT to detect naturally occurring mastitis under field conditions.

UH1-011-002

Milk microbiome assessed through 16S rRNA sequencing during antimicrobial treatment of mastitis - A Randomized Clinical Trial

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Objectives: Specific aims were: 1) evaluate the effect of antibiotics on cure of culture negative and Gram-negative bacteria associated clinical mastitis; 2) use next generation sequencing to assess the microbiome of milk samples from mastitic quarters and compare it to the microbiome of ipsilateral healthy quarter of cows diagnosed with mild or moderate mastitis caused by either a Gram-negative pathogen or with no bacterial growth on conventional aerobic culture; and 3) evaluate the over-time effect of prolonged antibiotic therapy on microbial profiles of mastitic milk.

Materials and Methods: Cows diagnosed with clinical mastitis with a Gram-negative pathogen or no visible growth on on-farm culture ($n=103$) were randomly allocated into Treatment group, which received 5 intramammary infusions of ceftiofur hydrochloride at 24-h intervals on the affected quarter, or Control. Serial samples were collected before intramammary infusion of antibiotics on days 1, 2, 3, 4, 5, 8, 10 and 14 after enrollment from the affected quarter and an ipsilateral quarter for microbiome analysis. The V4 hypervariable region of the bacterial 16S rRNA gene was amplified from genomic DNA by PCR and sequenced using the Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA). Sequences were processed via the MiSeq Reporter version 2.5 and QIIME version 1.7.0-dev, for classification of reads, quality filtering, and calculation of number of OTUs, Chao1, and Shannon diversity indexes.

Microbiome changes occurring over time and in response to intramammary antibiotic therapy were analyzed at the phylum and family levels using JMP Pro 11. The mean relative abundance in healthy quarters was used as reference for calculation of fold-changes. Comparison of the microbiome was performed using response screening analysis to assess the effect of clinical mastitis on the relative abundance of bacterial families. P-values were adjusted for false discovery rate.

The GLIMMIX procedure of SAS was used to assess the effects of clinical mastitis and intramammary treatment in OTU numbers, Shannon, and Chao1 indexes.

Results: The relative abundance of bacteria from the phylum Proteobacteria was greater ($P < 0.01$) in the milk from mastitic quarters infected by *E. coli* and *Pseudomonas* sp. compared with that of healthy quarters, driven mostly by greater abundances of Enterobacteriaceae ($P < 0.001$) and Pseudomonadaceae ($P = 0.03$). The average abundance of Firmicutes, Actinobacteria, Bacteroidetes, Tenericutes, Chlorobi, and the combination of remaining phyla was greater ($P < 0.05$) in healthy compared with mastitic quarters.

As opposed to cases of clinical mastitis associated with specific pathogens, major shifts in bacterial populations were not observed in the milk of mastitic quarters classified as negative culture by standard laboratory methods.

Intramammary treatment with ceftiofur hydrochloride did not improve clinical and bacteriological cures of mastitis compared with untreated controls. In cows diagnosed with *E. coli* associated mastitis, the mean relative abundance of Enterobacteriaceae decreased from study day 1 to 14 (62.6 vs. 9.7%) and no differences in the rate of change of this bacterial group were observed between groups that received intramammary treatment with ceftiofur hydrochloride or control. Overrepresentation of individual families and defined time-related changes in milk microbiome were not observed in cows affected by clinical mastitis associated with culture negative. Moreover, intramammary treatment with ceftiofur hydrochloride did not result in major shifts on milk microbiome from mastitic quarters in the culture negative group.

Conclusions: Next generation sequencing revealed significant differences in the metagenome of healthy and mastitic quarters from cows diagnosed with Gram-negative pathogens. However, the bacterial profile of healthy and mastitic quarters from cows diagnosed with no bacterial growth under aerobic conditions did not significantly differ.

There were no significant differences in clinical cure or relative abundance of the most abundant bacterial family from mastitic samples between treatment and control groups in any of the bacterial groups evaluated.

UH1-011-003

The effect of sampling techniques on microbial species in quarter milk samples

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Objectives: Milk samples give important knowledge on the aetiological agent causing mastitis. Bacteriological results of the milk samples are affected by many factors like sampling technique or the amount of bacteria in the milk.

The purpose of this study was to determine the effect of two different milk sampling techniques on bacteriological results from a PCR mastitis assay. Two different milk sampling techniques were evaluated: conventional manual aseptic sampling by milking (group A) and experimental sampling technique via the teat canal using a sterile cannula (group B).

Materials and Methods: The study was carried out on 44 commercial dairy farms during routine calls of the Production animal hospital of the University of Helsinki, Finland, from March 2011 to April 2013. A cow was included in the study if the Californian mastitis test (CMT) score in the milk of the affected quarter was ≥ 3 on a scale from 1 to 5. A case with clinical signs of mastitis in the milk of the quarter was recorded as clinical mastitis (CM) and a case with no clinical signs but an increased CMT score (≥ 3) as subclinical mastitis (SCM). A total of 88 cows were sampled and the total number of sampled quarters was 150.

The milk sampling technique was as follows: The teat was cleaned by removing visible dirt. Some squirts of milk were discarded and then milk was tested with CMT. Teat was disinfected with cotton wool moistened with 70% alcohol three times. Conventional samples (sample A) were first drawn to a 10-ml plastic milk tube (Linkoputki Plastone without preservatives, Mekalasi, Finland). Teat end was then disinfected again

and a sterile disposable teat cannula (Teat cannula, Vetcare, Finland) was pushed through the teat canal and sample B was taken to a 10-ml plastic milk tube. Somatic cell count (SCC) was measured with an electronic cell-counter DCC (DeLaval International AB, Sweden) in the laboratory (Production animal hospital) within six hours. Bacteriological examination of the samples was carried out with a Real-Time PCR assay (PathoProof™ Mastitis Complete-16 Kit, Thermo Fisher Scientific Ltd, Finland). The statistical analysis (chi, kappa) was done with Stata IC.

Results: In total, 100 SCM samples (SCC in average 1 249 000 cell/ml) and 50 CM samples (SCC in average 2 327 000 cell/ml) were included. SCC result was missing from 22 samples. Samples of group A comprised more microbial species than samples of group B ($p=0.00$). Samples of group A comprised 18% less samples with one species only than those in group B. More samples with 3 and ≥ 4 species detected were found in group A than in group B (samples with 3 species: 19 in A vs 6 in B; samples with ≥ 4 species: 14 in A vs 4 in B).

The microbial findings were different according to the sampling technique. The occurrence of many microbial species was lower in the milk samples taken with the cannula technique (group B). Coagulase-negative staphylococci (CNS) was a very common finding in both groups, and was detected more often in group A samples (83 samples) than in B samples (68 samples) (a decrease of 18%). As many as 35 A samples were positive for yeasts but only 15 B samples (a decrease of 57%). *Streptococcus dysgalactiae* was detected in 23 A samples and in 11 B samples (a decrease of 52%) and *Trueperella pyogenes/Peptoniphilus indolicus* was detected in 17 A samples and 6 B samples (a decrease of 65 %). The sampling technique did not affect the prevalence of *C. bovis*; 26 samples positive in group A and 23 samples positive in group B, kappa value (k)=0.63 indicating a substantial agreement between techniques. Kappa values of yeast ($k=0.14$) and *Trueperella pyogenes/Peptoniphilus indolicus* ($k=0.31$) indicated that cannula technique decreases the occurrence of those microbes in the samples.

Conclusions: Our results show that conventional milk sampling technique is more prone to contamination than the experimental cannula technique. More multi-species samples were found with the conventional technique. The cannula technique particularly reduced the occurrence of yeasts and *Trueperella pyogenes/Peptoniphilus indolicus*. Cannula technique improved the quality of quarter milk samples, but is not feasible as a routine method. Our results emphasize the importance of a careful aseptic technique in the conventional milk sampling, which is still the method of choice in daily work.

UH1-011-004

The interest of dairy farmers in mastitis diagnostics

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Objectives: The aim of the study was to determine the use of bacteriological diagnostics for clinical mastitis (CM), subclinical mastitis (SCM), and dry cow treatment (DCT) by Dutch dairy farmers. Additionally their interest in diagnostic tests and the characteristics they consider important were determined.

Materials and Methods: A telephone interview was conducted among 195 randomly selected Dutch dairy farmers, with a herd size of >20 adult

cows. For the telephone interview, a structured questionnaire was used, based on previously held face-to-face interviews with 20 non-randomly selected Dutch dairy farmers. The questionnaire consisted of a general part regarding background information of the farmer, udder health consultancy services on the farm and udder health characteristics. The specific part of the questionnaire discussed the farmers' current diagnostic and treatment approach, their interest in mastitis diagnostics, and the desired test characteristics of mastitis diagnostics for CM, SCM and DCT. The answers were registered in a database and were analysed using descriptive statistics and univariable and multivariable models.

Results: A treatment decision for CM was mainly based on clinical signs. For SCM and DCT the most used criterion was somatic cell count. Bacteriological culture (BC) is used by 34% of the farmers for CM cases, and 2% used the results for treatment decisions. If an on-farm test that would reveal the preferred treatment for CM within 12 hours would be available, 71% of the farmers indicated they would use it. For SCM and DCT, 55% and 34% respectively, indicated they were willing to use such an on-farm test, where 22% and 7% respectively, indicated to use BC currently.

Farmers were most often interested in 'the preferred treatment' as test outcome for CM (58% of the farmers) and DCT (15% of the farmers). For SCM the test outcome of interest was the causative bacterium (38% of the farmers). Farmers already using BC were 13.1 times (95% CI = 3.1-55.6) more likely to express their interest in the preferred treatment as test outcome for CM than farmers who do not use BC. Fourteen percent of the farmers were not interested at all in mastitis diagnostics for CM, increasing up to 28% and 55% for SCM and DCT, respectively.

Reliability was indicated most often as an important test characteristic for CM, SCM and DCT. Time-to-result (TtR) was for CM the second most important whereas for SCM and DCT, TtR was the least important. The median of the farmers indicated the preferred TtR as <8 hours for CM and 22-24 hours for SCM and DCT. Furthermore, a price of €10,- to €15,-, a hands-on-time of 7.5-10 minutes and <7% false outcomes were indicated as acceptable for CM, SCM and DCT.

Conclusions: Dutch dairy farmers are interested in mastitis diagnostics, specifically for CM. The use of mastitis diagnostics will likely increase when a reliable on-farm test that reveals the preferred treatment and with a suitable TtR is available.

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A cost-effectiveness analysis of on-farm culture for the treatment of clinical mastitis in dairy cows

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Objectives: The use of on-farm culture (OFC) as a means of selectively treating cases of clinical mastitis in dairy cows has been reported recently (Lago et al., 2011) and is increasingly being advocated as a rational approach to reducing the use of antimicrobial drugs on dairy farms. The purpose of this research was to use probabilistic sensitivity analysis to investigate the cost-effectiveness of OFC. A particular aim was to identify scenarios when OFC was likely to be cost-effective and, therefore, the types of herds for which an OFC approach could reasonably be recommended.

Materials and Methods: The model used was an adaptation of a previously published cost-effectiveness model (Down et al., 2013), with the addition of OFC-specific parameters based on previous research (Lago et al., 2011). A stochastic Monte Carlo model was implemented to repeatedly simulate the cost of a case of clinical mastitis that could be treated according to two different methods; i) a standard approach (3 tubes of intramammary antibiotic), ii) an OFC programme as described by Lago et al., (2011). Model parameters were based on values taken from the current literature where possible and 5000 model iterations after convergence were used.

Spearman rank correlation coefficients were used to evaluate the relationships between model parameters and net cost effectiveness of the different treatment approaches. Further scenario analyses were performed to identify key parameters that determined the probability that OFC was more cost-effective than the standard approach.

Results: The Spearman rank correlation coefficients revealed that the proportion of culture-negative cases and the difference in bacteriological cure rates had the largest impact on the probability that the OFC approach was more cost-effective than the standard approach. The magnitude of these values suggested that OFC may not be cost effective in a considerable number of dairy herds. Other model parameters that were correlated with the difference in cost-effectiveness were the milk withdrawal period, the cost of drugs, the time taken to treat the cow (and culture) and the rate of transmission from an infected cow to an uninfected herdmate.

The scenario analysis indicated that the OFC approach was most likely to be cost-effective when the proportion of culture-negative cases was at least 53% and when the bacteriological cure rate was not more than 5% poorer in the OFC group compared to the standard treatment group. It was also evident that if the bacteriological cure rate was approximately 11% worse in the OFC group than in the standard treatment group, then the proportion of culture-negative cases would need to be at least 79% to make OFC cost-effective.

Conclusions: The results of this study indicate that the proportion of culture -ve cases and the bacteriological cure rate have the greatest impact on the probability that an OFC approach would be more cost-effective than a standard approach. The OFC approach would be most suitable for herds in which Gram -ve pathogens are responsible for most clinical mastitis and where the treatment of cows according to the results of an OFC approach results in minimal reductions in the bacteriological cure rates. Results suggest caution should be exercised in using OFC without a transparent message that the method will probably not be cost-effective for many herds.

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Discriminating *Staphylococcus aureus* isolates from other common mastitis pathogens in dairy cows with scent dogs

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Objectives: The objective of this study was to proof that bacteria causing mastitis in dairy cows can be identified by their specific odor. Specifically we set out to train dogs to discriminate the odor of *Staphylococcus aureus* against five other common mastitis causing agents. The study included two experiments. First we tested if dogs can discriminate *Staph. aureus* in the headspace of blood agar plates growing *Staph. aureus* colonies. In the second experiment the dogs had to identify the odor of *Staph. aureus* in milk samples spiked with *Staph. aureus* or the other infectious agents, respectively.

Materials and Methods: 10 privately owned dogs of various breeds and both sexes were trained in this study. For the first experiment bacteria were isolated from milk samples from cows with clinical mastitis and cultured on Columbia Agar with 5% sheep-blood for 48 h. Identification of infectious agents was by their morphology and was confirmed by MALDI-ToF. Until use, strains were stored at -20°C in cryotubes. Before dog training or testing the infectious agents were again cultured on Columbia Agar with 5% sheep-blood. A cotton swab was placed in the lit of the plate to absorb the specific odors. For the second experiment only *Staph. aureus*, *Strep. uberis* and *Enterococcus* spp. were used. Bacteria were concentrated (1012Cfu/g and frozen at -20°C in cryotubes. For dog training and testing the bacterial concentrate was solved in 2 ml fresh bulk tank milk with SCC 120 000/ml. The cotton swabs or milk sample were placed into a 1 liter sterile plastic buckets with perforated lits. The tests for each experiment included 10 test trials. For one trial a dog had to discriminate one sample containing *Staph. aureus* against nine samples containing other infectious agents. Every dog tested 100 samples.

Results: Eight dogs completed the training and took part in the test of the first experiment. The training duration in minutes varied from 111 – 290 min (163.4 ±70.2min). Overall sensitivity for identifying *Staph. aureus* in the head space of agar plates against five other common mastitis causing agents (i.e., *Escherichia coli*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Pseudomonas aeruginosa* and *Candida albicans*) was 91.3% and specificity was 97.9 %. Four dogs did not make any mistakes. Breed or sex of the dog had no influence on the performance. Six dogs were included in the second experiment. In bulk tank milk the dogs could identify the samples with *Staph. aureus* against *Strep. uberis* and *Enterococcus* spp. with a specificity and sensitivity of 82% and 87%, respectively.

Conclusions: In this study we demonstrated that *Staph. aureus* can be identified by means of a specific odor in two substrates. Further research is warranted to proof that odor diagnostic either with technical methods such as gas chromatography or electronic noses devices or biosensors such as trained animals (e.g., rats, bees or dogs) could be a valid and practical diagnostic tool to identify mastitis pathogens.

UH2-012-003

Potentials and challenges for diagnosis of contagious udder pathogens with realtime PCR tests from samples at milk recording

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Objectives: Accurate diagnosis is necessary to control contagious udder pathogens in dairy herds. Since 2009 Danish farmers can order PCR analysis of routinely taken milk recording samples. The PCR test has higher sensitivity for detection of *Staphylococcus (S.) aureus* and *Streptococcus (S.) agalactiae* than bacteriologic culturing, but is carried out on non-aseptically taken samples. Thus, carry-over between cows and false positive results due to contamination may occur. Our objectives were to investigate carry-over effects between consecutively milked cows and the effect of teat preparation on PCR test results for *S. agalactiae* and *S. aureus*.

Materials and Methods: A total of 1,199 dairy cows from six herds infected with *S. agalactiae* and *S. aureus* were included in the study. Bronopol preserved composite milk samples were taken at the routine milk recording between March and May 2011 after the farm personnel had carried out their routine pre-milking practices. All samples were tested using the PathoProof Mastitis PCR assay and results recorded as cycle threshold (Ct)-values. Aseptically sampled quarter foremilk were subjected to bacteriological culturing for *S. aureus* and for *S. agalactiae* in 50% randomly selected cows, i.e. all cows milked at every second milking unit. Data on parity, somatic cell counts, days in milk, and kg energy corrected milk were obtained from the Danish Dairy Cattle Database.

Logistic regression including herd as random effect was used to investigate the effect of teat disinfection on *S. aureus* PCR positivity. Milking order was available from four herds. The effect of milking order on *S. agalactiae* PCR positivity was investigated applying logistic regression with generalized estimating equations. The effect of the Ct-value for *S. aureus* of the previously milked cow on the Ct-value of the subsequent cow was investigated with a multinomial logistic regression model. Five cut-off categories for PCR Ct-values were chosen: 0-31.3; 31.4-33.9; 34.0-37; 37.1-39.9; and 40.

Results: Teat preparation including teat disinfection reduced the odds of being PCR positive for *S. aureus* (OR 0.75, Mahmmoud et al. 2013), but not for *S. agalactiae*. Carry-over in terms of correlated PCR results (Ct-values) of cows milked consecutively occurred (Mahmmoud et al. 2014). The correlation was 13%, 11%, 9% at *S. agalactiae* Ct-value cut-offs 39, 37, and 34, respectively.

The multinomial logistic regression model showed that the Ct-value category for *S. aureus* of the preceding cow had a large impact on the Ct-value category of the subsequently milked cow. If the previously milked cow was classified as PCR negative (Ct-value 40), the subsequently milked cow at the same milking unit was approximately three times more likely to be PCR negative (prevalence 60%) in comparison to cows that were milked after a cow in the PCR category 0-31.3 (prevalence 20%).

Conclusions: Milk samples for PCR diagnostics should preferably be taken aseptically, especially if information on milking order and infection status of the previously milked cow is unavailable. Composite milk samples from milk recording can be used for PCR diagnostics of *S. agalactiae* and *S. aureus* if teats are disinfected and the milking order is known. Teat disinfection will reduce false positive results for *S. aureus*. Carry-over effects for *S. agalactiae* and *S. aureus* occur and have to be addressed by accounting for milking order, repeated testing of positive cows milked subsequent to a positive cow and by considering other inflammation markers.

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Efficacy of Topical Treatment of Udder Cleft Dermatitis

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Objectives: Udder Cleft Dermatitis (UCD) is characterized by lesions of the skin between the front quarters or between the front quarters and the abdominal wall. UCD can be categorized into two groups, depending on the integrity of the skin. Mild cases are characterized by crusts, sebum, erythema or transudate, but with an intact skin, whereas severe cases are characterized by open wounds combined with pus and necrosis. To date no effective treatment has been proven which reduces the duration of UCD. The objective of this study was to investigate the efficacy of two different topical treatments for mild and severe cases of UCD.

Materials and Methods: A randomized clinical trial was performed on 8 Dutch dairy herds with a high prevalence of UCD. At T0 all cows present were inspected for signs of UCD and photos were taken from the affected animals. The lesions were photographed together with a ruler, so the length of the lesion could be measured. The photos were judged by an external wound expert who classified the affected animals in three different groups: mild lesions, severe lesions < 5 cm or severe lesions ≥ 5 cm.

Within each herd mild cases were randomized into a treatment group or control group, whilst severe cases were stratified into < 5 cm and ≥ 5 cm before they were randomized into treatment or control.

Cows that were included in the study were identified using different colored leg bands corresponding to treatment groups (mild or severe). Cows in the control groups were not identified, because they were not treated by the farmer. All farmers were supplied with treatment protocols and trained in how to treat the affected animals after milking. Treatment of the mild cases consisted of rubbing the affected area every other day with Cavulon®, a small foam wipe that should prevent skin breakdown. Severe cases were treated every day with 10 gram Botop®, an enzyme alginate. Cows were checked and photographed once a week and photos were sent to the wound expert who was blinded to the treatment and control groups. At the end of the 12 week study period cows could be cured, improved, unchanged, or deteriorated.

Results: Within-herd prevalence at T0 varied between 10% and 46%. Five out of eight herds had more mild than severe cases. A total of 206 animals were included in the study and were followed for a period of 12 weeks. From all affected animals 113 cases were mild (57 treated, 56 controls), 47 cases were severe < 5 cm (25 treated, 22 controls) and 46 cases were severe ≥ 5 cm (25 treated, 21 controls).

In the group of the mild cases no significant difference was observed between the treated and the control animals. After 12 weeks 36.8% of the treated and 37.5% of the control animals were cured. Animals with severe lesions < 5 cm showed significant differences. In the treated group 28% of the cases were cured and 32% of the cases changed from severe to mild (improved), compared to 13.6% and 4.5% respectively in the control group ($P < 0.01$). In the treated severe cases ≥ 5 cm group 36% of the cases were improved compared to only 4.7% of cases improving in the severe control group ($P < 0.05$). Only a 2% cure was observed after 12 weeks in both the treated and control group. Overall results of the severe cases (<5 cm and ≥5 cm) showed 18% cure and 34% improvement in the severe treated group compared to 12% cure and 5% improvement in the severe control group.

Conclusions: No significant differences were seen in animals with mild lesions that were treated with Cavulon® compared to the control group. This might be due to the small format of the wipe which made it difficult

to treat the affected area. Treatment of the severe cases with Botop® however proved to be a successful therapy. Treated severe cases < 5 cm had a significantly higher cure rate than the controls, whereas the treated severe cases ≥ 5 cm showed significant improvement during the study period of 12 weeks, since 12 weeks was too short for total recovery. To our opinion this is the first study showing an effective treatment for severe cases of UCD.

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The Efficacy Of A Novel Combination Intramammary Treatment For Mastitis In Dairy Cows In New Zealand

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Objectives: The two most common udder pathogens found in clinical mastitis in New Zealand dairy cows are *Staphylococcus aureus* and *Streptococcus uberis*. Traditionally, cloxacillin is recognized as the choice of treatment for the former and penicillin for the latter, which leads to some challenging therapeutic decisions, further complicated by the presence of resistance in the former pathogen. This large scale field study was designed to test the non-inferiority of a novel combination intramammary product containing penicillin and cloxacillin under field conditions in New Zealand, with regards to bacteriological cure and clinical cure proportions.

Materials and Methods: Clinical cases of mastitis were sourced from 30 spring-calving dairy farms in the Southland region of New Zealand. Affected quarters were infused three times at 24 hourly intervals with either the novel combination product containing 1g penicillin and 200mg cloxacillin, or a reference product containing 200mg oxytetracycline, 100mg oleandomycin, 100mg neomycin and 5mg prednisolone. Cows were enrolled when a farmer detected a case of clinical mastitis. Milk samples were collected for microbiological culture immediately before treatment (day 0) and on days 9, 16 and 23 after enrolment. A total of 235 quarters treated with reference product and 223 quarters treated with the novel combination product were eligible for per protocol analysis. Non-inferiority was assessed by calculating the difference in cure rates between the two products and constructing a 95% confidence interval around the difference. The confidence interval around the difference was inflated by the square root of the variance inflation factor (VIF) to account for herd level clustering. The non-inferiority margin (δ) was 20% for both bacteriological cure and clinical cure. The effect of covariates on bacteriological cure and clinical cure rates were assessed using Generalizing Estimating Equations (GEE) to account for herd level clustering.

Results: A total of 93 cases (20.3% of all cases) were recorded as having no clinical cure during the follow up period. Clinical cure occurred in 79.6% of cases treated with the reference product, and 79.8% of cases treated by the novel combination product. The clinical cure proportion was 0.3% (-11.2%, -12%) higher for clinical quarters treated with the novel combination product, which was demonstrated to be non-inferior to the reference product with regards to clinical cure rates, as the lower confidence interval of the difference between treatments did not extend beyond the lower limit δ of -0.20.

Bacteriological cure occurred in 57.2% of cases treated with reference treatment and 65.7% of cases treated by the novel combination treatment. The bacteriological cure proportion was 8.51% (95%CI:

-1.7%, -21.8%) higher for clinical quarters treated with the novel combination product. The novel combination product was demonstrated to be non-inferior to the reference product with regards to bacteriological cure rates as the lower confidence interval of the difference between the treatments did not extend beyond the lower limit δ of -0.20. The adjusted cure rate for *S. aureus* was 17.6% higher for quarters treated with the novel combination product, however this difference was not significant ($P>0.05$).

In addition, the novel combination product reduced somatic cell counts during the follow up period ($p=0.053$). Pathogen type was a significant predictor on the bacteriological cure proportion, and quarter score at enrolment was a significant predictor on the clinical cure proportion.

Conclusions: The novel combination product was demonstrated to be non-inferior to the reference product with regards to both bacteriological cure proportion and clinical cure proportion. Under field conditions where Gram positive mastitis pathogens are predominant this combination of antimicrobials is an efficacious treatment.

Thus, this novel combination intramammary product provides appropriate and effective treatment for both *S. uberis* and *S. aureus* under New Zealand field conditions. Using a combination product where there is a risk of antimicrobial resistance also reduces the risk of developing resistance within a pathogen class.

Comments: This study was funded by Virbac (NZ) Ltd (formerly Stockguard Laboratories Ltd).

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Evaluation of Intramammary Infusion of Lactic Acid Bacteria and Therapeutic Effects on Mastitis in Dairy Cows

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Objectives: Development of alternative therapeutic approaches to antibiotics is of increasing concern for control of mastitis. The objective of the study was to characterize the cellular and immune responses of mammary gland by intramammary infusion of lactic acid bacteria (LAB) in dairy cows, and to evaluate the therapeutic effects of LAB to lactating cows with mastitis.

Materials and Methods: Twelve Holstein cows were used to analyze the cellular and immunological effects of mammary glands by infusion of *Bifidobacterium breve* (*B. breve*, heat-killed 3×10^9 cfu) was used. Thirty-eight cows with subclinical mastitis were used for evaluation of therapeutic effects of *B. breve*. Milk samples were collected from the quarters prior to infusion and 1, 5, 7, 14 and 21 days post infusion. Somatic cell counts (SCC), milk composition, N-acetyl-glucosaminidase (NAGase) activity and mastitis-causing pathogens, lactoferrin (Lf), immunoglobulins in quarter milk were determined. Chemiluminescence response in milk, cytokine mRNA (IL-1 β , TNF- α , IL-6, 8, NF- κ B) on milk leukocytes and their cytokines were determined.

Results: Significant increases in SCC, NAGase activity were found at 1-2 days post infusion and their values decreased after 3-5 days post infusion. Ten to 30 fold increases in numbers of SCC were found in milk from quarters at 1-2 days after infusion of LAB. Significantly increased concentrations of Lf, IgG and IgA, and increased mRNA

expression of cytokines IL-1 β , TNF- α , IL-6, 8 on milk leukocytes were found in cows at 1-3 days after intramammary infusion. The therapeutic trials of intramammary infusion of LAB were evaluated in dairy cows with subclinical mastitis: bacteriological cure rates (<300 cfu/ml) were 64.7 % (11/17) for environmental streptococci, 66.7% (8/12) for CNS, 0-20% (1/7) for *Staph. aureus* at 7-14 days post infusion. Mean SCCs in 14 quarter milk with no bacteria growth were significantly decreased at 14 days post infusion.

Conclusions: Intramammary LAB infusion promotes the clearance of the pathogens causing mastitis from quarters and alleviates the local infection in affected quarters from cows with subclinical mastitis. Intramammary infusion of LAB may be a possible approach for non-antibiotic treatment for mastitis.

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Application of Multilocus Sequence Typing in *Streptococcus agalactiae* isolated from Bovine Mastitis in China

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Objectives: To investigate the occurrence and distribution of *Streptococcus agalactiae* (*S. agalactiae*) from bovine clinical mastitis and subclinical mastitis in different regions of China and to assess the molecular epidemiology and population structure of the Chinese isolates.

Materials and Methods: Twenty-three isolates of *S. agalactiae* were recovered from the milk of cows with bovine Clinical and subclinical mastitis in four provinces (Beijing, Anhui, Hebei, Heilongjiang) of China between 2014 and 2015. The identification of *S. agalactiae* was done by Edward Medium (modified), biochemical method and Polymerase Chain Reaction (PCR) with 16S rRNA gene sequence. Application of multilocus sequence typing (MLST) was to establish the clonal groups. Fragments (459 to 519 bp) of seven housekeeping genes (*adhP*, *PheS*, *atr*, *glnA*, *sdhA*, *glcK*, *tkt*) were amplified by PCR for each strain and sequenced. The combination of alleles at the seven loci provided an allelic profile or sequence type (ST) for each strain. The entire *S. agalactiae* MLST database and eBURST program were used to analyze global clonal complex (GCC).

Results: Fifteen *S. agalactiae* isolates were recovered from the 121 cases of clinical mastitis and eight *S. agalactiae* isolates were recovered from the 87 cases of subclinical mastitis. Ten *S. agalactiae* isolates were recovered from Beijing, Six from Hebei, five from Heilongjiang and two from Anhui. All the twenty-three *S. agalactiae* isolates from bovine mastitis in China were identified by MLST to possess a similar allelic profile ST-103. The eBURST analysis of *S. agalactiae* MLST data showed that ST-103 was in GCC103, which remained as a distinct group.

Conclusions: *S. agalactiae* was the most common pathogen isolated from the bovine Streptococcal mastitis in China. ST-103 was emerged as a highly prevalent clone in bovine clinical and subclinical mastitis among different regions of China.

Comments: This study provided useful information for further studies on the genotyping and evolutionary research for *S. agalactiae* strains with global diversity. The assay will be useful for the development of vaccines against mastitis caused by *S. agalactiae*, as well as for the

treatment and control of bovine mastitis. This research was supported by No. 2012BAD12B03, No. 313054, No. 20120008110042 and No. 31572587.

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Risk factors for calf mortality and poor growth of dairy heifer calves in Germany

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Objectives: The objectives of this study were to investigate calf mortality rates, the average daily weight gain till weaning and factors associated with the risk of mortality and of poor growth in young dairy replacement heifers in Germany on a herd-level bases.

Materials and Methods: A case-control study was performed in 50 herds, which were selected based on acceptance to participate in the study and were located in northeast Germany. The experimental unit was the herd. Each herd was visited once between 2012 and 2014. A questionnaire on farm management practices, morbidity and calf mortality was completed by in-person interview. Furthermore colostral, blood und faecal samples were collected to check for colostral quality, failure of passive transfer (FPT), nutritional status and faecal pathogens causing neonatal calf diarrhea. Heart girth measurements were conducted around weaning (12th week) to assume average daily weight gain (ADG). Additionally a score concerning calf welfare was determined. The epidemiological associations between these factors with calf mortality and with poor growth were estimated by using two logistic regression models. In the first step all candidate variables were screened in univariable analysis and those associated with the outcome variable at $p \leq 0.20$ ($n=14/23$) qualified for multivariable analysis, but just 7 candidate variables each were selected. The initial multivariable models were reduced using a manual stepwise backward procedure, with $p < 0.1$ as criterion for retention. Variables excluded during the reduction were re-entered one by one after all remaining variables were significant ($p < 0.05$).

Results: The median calf mortality risk in calves 1–6 month of age was 5.0% (Range: 0.0–17.7%). The factors significantly associated with high calf mortality (>5.0%) were the application of Halofuginon ($p < 0.05$, OR: 6.7) and the access to hay in the first week of life compared to those offered no hay before weaning ($p < 0.05$, OR: 0.2). In the multivariable logistic regression model the factors application of Halofuginon ($p < 0.01$, OR: 10.0) and a high rate of calves with FPT ($p < 0.05$, OR: 8.1) explained 37.6% of the variation between herds with high and low calf mortality.

On herd-level the median ADG in calves 3 month of age was 675 grams per day (Range: 414–1027 g). The risk factors significantly associated with poor growth were offering hay before weaning ($p < 0.05$, -142 g), collecting first colostrum later than two hours after parturition ($p < 0.05$, -142 g), the amount of concentrates consumed at weaning ($p < 0.01$, +130 g per kg concentrates), changing the bedding in the calf pen at least once every two weeks ($p < 0.05$, -96 g), relocating calves more than twice from birth till weaning ($p < 0.01$, -93g), feeding less than 3.0 liters of first colostrum ($p < 0.05$, -88 g) and leaving the maternity pen more than two hours per day unattended ($p < 0.05$, -84 g). In the multivariable logistic regression model the factors amount of concentrates consumed at weaning ($p < 0.001$, +160 g per kg concentrates), relocating calves more than twice from birth till weaning ($p < 0.001$, -119g) and having an incidence of milk fever under 5% ($p < 0.01$, -115 g) explained 72.8% of the variation in growth of calves around weaning.

Conclusions: These results indicate that management practices in dairy farms rearing their own replacement heifers have an impact on calf mortality and on poor growth till weaning. They should be taken seriously concerning calf health and animal welfare.

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Calving characteristics and physical examination parameters associated with abnormal underlying physiology in newborn beef calves

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Objectives: Abnormal physiology resulting from dystocia is associated with failed transfer of passive immunity, morbidity, and mortality in newborn calves. Modified Apgar scores developed to identify compromised calves are not widely used, apparently due to lack of practicality and inconsistent associations with underlying blood physiology. The objective was to evaluate associations of underlying blood physiology with physical examination parameters and calving characteristics to determine key parameters indicative of acidosis in newborn beef calves.

Materials and Methods: Data were collected from 77 dystocic and non-dystocic calves born on a large commercial cow-calf operation in Alberta, Canada (Spring of 2014). Blood parameters at 10 min and 24 h after birth were compared to calving characteristics and at-birth physical examination parameters. Parametric blood parameters were assessed using Student's t-test for dichotomous outcomes and ANOVA with Tukey-Kramer post hoc test for multiple comparisons. Wilcoxon test for dichotomous outcomes and Kruskal-Wallis with Dunn post hoc test for multiple comparisons were used for non-parametric variables. Repeated measures ANOVA was used to test main effects and interactions for all significant calving characteristics and at-birth physical examination parameters. Unconditional associations of blood parameters with continuous at-birth calving characteristics and physical examination variables, as well as lactate, were evaluated using Pearson correlation coefficients.

Results: Traditional Apgar score parameters, heart and respiratory rate, were not associated with pH or lactate ($r < 0.25$); however, lactate was strongly correlated with pH ($r = -0.86$). Mucous membrane colour (another traditional parameter) was not associated with pH ($P = 0.07$) but was associated with lactate ($P = 0.002$). Tests of muscle tonicity and reflexes, namely inability to completely withdraw the tongue when pinched and weak suckle reflex, were associated with both decreased pH and increased lactate ($P < 0.05$ for each). Calves born to primiparous dams or with increased calving difficulty also had decreased pH and increased lactate ($P < 0.05$ for each). Differences in pH between at-birth categories resolved for all parameters by 24 h after birth, except tongue withdrawal ($P = 0.04$).

Conclusions: Traditional Apgar parameters, heart and respiratory rate and potentially mucous membrane colour, may not consistently identify compromised calves with acidosis. Indicators of muscle tonicity and reflexes, such as degree of tongue withdrawal and strength of suckle reflex, as well as calving ease and parity, should be further examined. Additionally, hand-held meters to determine blood lactate concentration may be a useful on-farm tool for identification of acidotic calves.

YS1-048-003

The impact of colostrum on the microbiological health of the developing bovine intestinal tract

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Objectives: Enteric disease is a major cause of morbidity and mortality in young calves. In recent years, studies have showed that beneficial commensal microbes contribute to intestinal health by supporting epithelial function, host metabolism and immune development. Microbial colonization begins with the acquisition of pioneer organisms from the dam. After this, composition of the microbiome varies but the most desirable trajectory is towards richness and diversity. In this study we studied the impact of colostrum on the bovine neonatal microbiome during the pre-weaning period.

Materials and Methods: A total of 12 healthy male Holstein calves were separated from the cow immediately following birth, and fed 4 liters of aseptically collected, high quality colostrum. All calves were housed, monitored, and fed separately for the remainder of the experiment. A group of 3 animals were euthanized for necropsy, and intestinal samples collected before colostrum administration (day 0) and progressively during the rest of the trial (days 3, 7 and 21). Intestinal mucosal and luminal samples were collected from the duodenum, jejunum, ileum and colon. The microbiome of these samples were analyzed using PCR with V3-V4 16S rRNA gene marker and the current generation MiSeq (Illumina) sequencing technology. Total microbial richness was assessed through rarefaction analyses and diversity was determined by Shannon's diversity indices, as well as the change in relative abundance of the important bacterial species.

Results: The intestinal samples were different from the stomach samples, especially comparing the digesta to scraping samples. The digesta samples had a large proportion of sequences unclassified at the family level, as well as rare families that had <1% abundance each. While Prevotellaceae was not in large abundance in the digesta samples, it was found in higher abundance in the proximal jejunum scrapings. Burkholderiaceae was found in some digesta from the jejunum and ileum and in large abundance in the duodenum, jejunum, and ileum scrapings. In the intestines and stomach, Enterobacteriaceae decreased over time, while Comamonadaceae increased over time.

Colostrum samples had a large proportion of sequences unclassified at the family level, and rare families that had <1% abundance each. Bacteroidaceae, Caulobacteraceae, Comamonadaceae, Flavobacteriaceae, Pseudomonadaceae, and Xanthomonadaceae all decreased over time, Lachnospiraceae maintained abundance, and Ruminococcaceae increased over time. The pharynx scrapings showed a decrease in Pasteurellaceae, Enterobacteriaceae, Clostridiaceae, and Streptococcaceae, with an increase in Comamonadaceae over time. Clostridiaceae was found in the pharynx at day 1 but no Clostridiaceae were found in colostrum at any sampling time. On day 7, a large proportion of Porphyomonadaceae, typically sugar fermenters, were found. Pasteurellaceae contains many mucosal commensal bacteria. The udder and vaginal scrapings showed a large amount of diversity.

Conclusions: Gradual changes in gut microbiota were seen with age and indicated a developing microbial ecosystem consistent with previous reports. The unique aspect of this study design and resultant data, is the particular focus on the contribution of colostrum to development of a balanced microbial ecosystem in the gastrointestinal system of the young calf. In future studies this model will be employed to examine the impact of various colostrum handling and management strategies on the developing microbiome.

YS1-048-004

Associations and agreement between deep nasopharyngeal swab and non-endoscopic bronchoalveolar lavage bacterial culture results in preweaned calves

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Objectives: A broncho-alveolar lavage (BAL), performed with a BAL-catheter without endoscopic guidance, is a practically achievable alternative to sample the deep airways in a large number of calves in a limited time frame. Sample quality and agreement with deep nasopharyngeal swab (DNS cultures) are unknown.

Therefore, the objective of the present study was to determine isolation rates of respiratory bacteria, agreement and associations between DNS and BAL samples.

Materials and Methods: A cross-sectional study was performed on preweaned calves with bovine respiratory disease (BRD) (n= 144) from 11 commercial herds (2 veal, 9 beef). DNS and BAL samples were taken from each calf and cultured to detect Pasteurellaceae and Mycoplasma spp.. Culture results were differentiated in a negative culture, polymicrobial culture, pure culture and a dominant culture. Isolation rates between DNS and BAL samples were determined by the McNemar chi-square test for correlated proportions. Agreement and associations between DNS and BAL samples were determined by kappa statistics and logistic regression.

Results: Isolation rates of all studied respiratory bacteria were not different between DNS and BAL samples, with the exception of *Histophilus somni* which was more likely to be isolated from BAL samples (P=0.01). BAL samples were less polymicrobial (P<0.001), more negative (P<0.001) and yielded more pure cultures compared to DNS (P<0.001). Agreement between DNS and BAL was poor for *H. somni* (κ : 0.16) and moderate for the other bacteria (κ : 0.48-0.58). A polymicrobial DNS did not increase the probability of a polymicrobial BAL result, but decreased the probability of isolating a pure culture from the BAL sample for *Mannheimia haemolytica* (P= 0.03) and *Pasteurella multocida* (P< 0.01), except for Mycoplasmata.

Conclusions: In conclusion, BAL samples taken without endoscope are less contaminated compared to DNS, which facilitates interpretation by both bacteriologist and clinicians. The discrepancy between the polymicrobial nature from the DNS compared to the pure culture or negative results from BAL in the same animal suggests that BAL catheter contamination by nasal and pharyngeal passage is less massive than previously believed.

YS1-048-005

The effect of Bovine Respiratory Disease on the Average Daily Gain of Calves in Finland

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Objectives: Bovine respiratory disease (BRD) is a common disease of calves causing economical losses and affecting animal welfare. In Finland BRD seem to have increased as the herd size and calf rearing units have increased. The objective of this research was to calculate the effect of BRD on the average daily gain of calves in Finland.

Materials and Methods: Medication data was collected from five calf rearing units in Finland. The study population was a convenience sample, criteria used to choose the farms were: farms were partners for slaughterhouse Atria, farms had similar infrastructure, the amount of medication used (two farms reported considerable and three farms slight medication) and willingness to participate.

Medication history was collected from 21 groups (3-6 groups per farm) in the year 2010. Calves were categorized to be either healthy, BRD, BRD with another disease or have only another disease. In addition, the frequency of antibiotic treatment for BRD was documented. Only data of the animals that survived until slaughter were included in the study. The final study population was 1031 calves: 358 healthy, 460 with BRD, 98 with other disease and 115 with BRD and another disease. 456 calves had not been given antibiotics, 385 calves had one antibiotic treatment, 190 calves two or more treatments. The length of one antibiotic treatment varied between 1-4 days. Weight was measured at the time of transfer to the finishing unit (mean age of 158 days, 5.3 mo.) and at the slaughter (mean age 584 days, 19.5 mo.).

The effect of other clinical diagnose than BRD, gender, breed, age and square term of age will be studied in a linear random model. Farm and patch of rearing calves will be included as random factors.

Mycoplasma bovis was first diagnosed in Finland in December 2012. At the time of the collected data (2010), it is assumed that BRD was caused by other pathogens than *M.bovis*.

Results: Average estimated daily live weight gain for healthy bull calves was 1040 g at the transfer. Estimated daily carcass gain was 547 g at the slaughter (n = 307). On the transfer, the average estimated daily live weight gain of calves that were not treated for BRD was 59 g (95% CI 31-86) more than the gain of calves that were treated twice or more with antibiotics (p = 0.000) and 24 g (95% CI 3-45) more than the gain of calves that were treated once (p = 0.024). The calves treated twice or more with antibiotics for BRD had 35 g (95% CI 12-58) less daily live weight gain than the ones treated once (p = 0.003). There was no interaction effect to the weight gain between antibiotics treatment for BRD and some other clinical diagnosis.

The average daily gain had no longer significant difference at the time of slaughter when comparing animals with treatment and no treatment for BRD.

Conclusions: Calves that have BRD and get at least two antibiotic treatments are clearly affected and their average estimated daily live weight is significantly lower at the time of transfer to the finishing unit. Animals treated only once had also lower average estimated daily live weight but it was less affected. By the time of slaughter there was no longer significant difference. Animals with previous antibiotic treatments for BRD, catch up during the finishing period and do not increase economical loss by lesser weight of the animals at slaughter.

YS1-048-006

Use of available national data to characterise calf survival in Irish dairy herds.

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Objectives: The Department of Agriculture, Food and the Marine (DAFM) maintains cattle traceability records on Animal Identification and Movement System (AIM). Irish dairy herds rely on replacement calves to provide the foundation of future productivity yet a high proportion of calves die on-farm in early life. The objectives of this study were to use AIM data to (1) determine mortality rates for calves based on herd of origin, (2) categorise herds based on calves born reaching age milestones (3) identify key risk factors present on AIM associated with calf survival and (4) assess potential of AIM data to generate a predictive model for calf survival.

Materials and Methods: Data relating to all calves born to dairy breed dams in 2013 and 2014 and respective herds of origin were identified on AIM and subjected to univariate and multivariate analysis. Specific calf level information included in the analysis was; date of birth, herd of birth, sex, breed, dam parity, movement history, date of death, age at death, disposal location. Specific herd level information included in the analysis was; herd location, total herd size, number of dairy cows in herd. Dairy herd expansion or contraction was determined by identifying changes in total cattle and dairy cow numbers in the years 2013, 2014 and 2015. Individual mortality rates (MR) were generated for all Irish dairy herds in 2013/14 by comparing total calves born to on-farm deaths and were grouped according to age at death (survival period) and geographical location of herd of origin. Herds were categorised according to life expectancy of calves born into a herd. Categories used to group herds for each survival period were high mortality (MR > 12%), medium mortality (6% < MR < 12%) and low mortality (MR < 6%). A data modelling logistic regression to identify risk factors that influence life expectancy of calves for three survival periods (0-21 days, 21-183 days, >183 days), as well as the impact and the effect of each factor into these pre-defined periods.

Results: The dataset examined comprised of 3,186,589 male and female calves born in 27,145 dairy herds. Birth pattern and mortality pattern was seasonal with 70.5% of deaths in calves born occurring in the first quarter. Mortality rates for age groups 0-21 days, 22-42 days, 43-92 days, 93-183 days and greater than 183 days were 3.92% (124971 calves), 0.85% (27119 calves), 0.78% (24755 calves), 1.01% (32120 calves) and 1.14% (36236 calves) respectively. Average on-farm deaths across all 26 Irish counties for all calves born for the duration of the data period examined was 10.55% in 2013 and 8.23% in 2014 respectively with a with a range of 6.61% to 13.97%.

13.8 % of herds nationally had a mortality rate in excess of 6% in calves under 3 weeks old. 745 herds (representing 2.7% of all herds and 2% of expanded herds) were identified with mortality rates in excess of 12% for 0-21 days category and a further 736 herds were in excess of 12% mortality for calves 21-183 days. Data modelling identified dam parity, calf sex, total number of farm to farm movements, total number of movements through a mart, total herd size, dairy herd size, breed, and date of birth as factors associated with calf survival (P < 0.05). Geographical location of herd was not identified as significant. Predictive value of the model was low as factors identified were only able to explain approximately 15% of data variability and are subject to bias and data quality considerations.

Conclusions: Mortality rates for dairy calves based on herd of origin were identified. Mixing of calves from multiple sources into herds in an era of herd expansion may mask a "herd of origin effect" unless analysis of this type is performed. High mortality rates indicate weaknesses in calf production systems within certain herds. The model generated provides proof of concept that data of this type has a value in development of methods to predict calf survivability and identify

factors associated with calf survival. The predictive value of the model was low due to data limitations but inclusion of further data streams may increase predictive power.

YS2-049-001

Development of a diagnostic tool predictive of the ability of newborn beef calves to consume colostrum within 4 hours

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Objectives: Newborn beef calf vigour is essential, as it helps ensure timely and adequate colostrum consumption required for successful transfer of passive immunity. Acidosis and asphyxia, often associated with dystocia, can negatively affect vigour. Unfortunately, Apgar scores designed to identify compromised calves are not widely adopted due to impracticality, inconsistent associations with underlying physiology, and unclear recommendations for intervention. Therefore, the objective was to develop a producer-friendly diagnostic tool predictive of a newborn calf's ability to consume colostrum in a timely manner.

Materials and Methods: Data were collected from 77 dystocic and non-dystocic calves born on a large commercial cow-calf operation in Alberta, Canada (Spring of 2014). Following birth, all calves were placed in individual maternity pens with their dams and monitored for 4 h. Calves failing to consume colostrum on their own within 4 h after calving were assisted in colostrum consumption. Blood parameters collected at birth were compared between calves that successfully sucked on their own before 4 h versus those that required assistance to consume colostrum. Multivariable logistic regression using backward stepwise selection was performed to determine associations of calving characteristics and physical examination parameters with the calf's ability to consume colostrum.

Results: The proportion of calves that sucked on their own before 4 h for unassisted, easy assists and difficult assists were 86, 61 and 36%, respectively. Calves failing to suck on their own had lower pH, HCO₃⁻ and base excess and higher lactate compared to their cohorts (P<0.05). Calving ease along with strength of suckle reflex, reaction to tongue pinch, mucous membrane colour, and ambient temperature were predictors of a calf's ability to suck on their own before 4 h (P<0.01). As a diagnostic tool for identifying calves unlikely to suck on their own, calving ease offered the highest sensitivity (89%) whereas suckle reflex had high specificity (98%). Suckle reflex alone was the most accurate method of predicting ability to consume colostrum (P<0.0001), as calves with a weak suckle reflex were 41.6 times less likely to suck on their own as compared to calves with a strong suckle reflex.

Conclusions: Measuring suckle reflex (weak versus strong) at birth is a quick, easy, and simple test that can be performed by producers to assess overall vigour of newborn beef calves. Calves with a weak suckle reflex should be assisted in delivery of colostrum, as suckle reflex was an excellent predictor of inability to consume colostrum in a timely manner. In addition, utilizing calving ease in parallel with suckle reflex may further improve the ability to predict calves unlikely to consume colostrum without assistance.

YS2-049-002

Associations between lung consolidation, bovine respiratory disease treatment and live-weight in UK veal cattle.

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Objectives: Estimates of bovine respiratory disease (BRD) prevalence on individual farms are usually based on mortality and morbidity (expressed as number of animals treated) data. However this relies on the accuracy of identification of clinical disease and will not include animals with subclinical disease. Examination of lungs at slaughter, combined with treatment records for BRD should provide a better estimate of the true prevalence.

The aim of this study was to examine the prevalence of lung consolidation in UK veal cattle at slaughter and the impact on live-weight at 6 months of age, and associations with treatments for BRD.

Materials and Methods: A total of 189 veal cattle aged between 177 and 240 days from two farms in the UK were examined at a commercial abattoir for evidence of lung lesions. Both farms utilised the same multivalent BRD vaccine protocol and had identical veterinary agreed treatment protocols for BRD.

Lungs were inspected on the slaughter line by the same investigator throughout the study period, using a combination of visual inspection and palpation to detect any tissue consolidation. The left and right cranial, cardiac and diaphragmatic lobes were scored individually as follows: 0: no consolidation in lobe, 1: up to 50% lobe consolidated, and 2: greater than 50% lobe consolidated. Because a lung was divided in 6 different lobes, the maximum total lung consolidation score was 6 x 2= 12. Animals were classified by total lung score: Normal/mild (score 0-1), Moderate (score 2-4) and Severe (score ≥5).

For each animal kill, information was collected from abattoir computerised records: official ear tag number, date of birth, producer name, net weight and carcass grade. Individual animal treatment history was collected from producer records: official ear tag number, treatment date and treatment reason. At the time of lung scoring, the study investigator was blinded to both kill and treatment data, but knew the producer's identity.

To account for variation in dead-weight due to variable age at slaughter, an estimated live-weight at 182 days was calculated from age and weight at admission to the units, live-weight at slaughter (assumed killing-out percentage of 52%) and age at slaughter.

Results: Mean estimated weight at 182 days was 221.2kg (sd=40.2kg).

130 out of 189 (68.8%) animals were identified as having at least one lung lobe up to 50% consolidated. There were 122 animals (64.6%) classified as having a Normal/mild lung score, 48 (25.4%) classified as Moderate and 19 (10.1%) as Severe.

Animals with Normal/mild lung scores had significantly higher (p<0.001) estimated mean weight at 182 days (230kg) than those with Moderate lung scores (203.1kg) and those with Severe lung scores (178kg).

Of all cattle, 42 (22.2%) received at least one treatment for BRD, with 15 (7.9%) receiving two or more treatments. In a linear regression model incorporating BRD treatment, age at arrival, weight at arrival and unit, animals with BRD were 16.4kg lighter (p=0.012) at 182 days.

Animals with Moderate scores had 2.63 times the odds (p=0.016) of having had a BRD treatment than those classed as Normal/mild. Animals classed as Severe scores had 5.20 times the odds (p=0.002) of having had a BRD treatment than those classed as Normal/mild.

Of the 48 animals with Moderate lung lesions, 33 (68.8%) had not received a BRD treatment; of the 19 Severe, 10 (52.6%) had not received treatment.

Conclusions: Both BRD treatment and lung consolidation were associated with a biologically significant lower live-weight at 182 days (six months)

Around two thirds of animals showed some lung consolidation at slaughter, yet only around a quarter were previously treated for BRD. This suggests that either the farmer chose not to treat certain animals with BRD or, as is more likely to be the case, the disease was not detected or was subclinical.

Comments: This study highlights the economic impact of reduced performance due to lung damage and that the current method of on-farm disease detection underestimates the true disease prevalence. The wider training of farmers in the use of BRD scoring systems, such as the Wisconsin Calf Health Score, may improve disease detection rates. New automated disease detection systems, based on changes in animal behaviour and the presence of pyrexia, should be evaluated as a potential tool for improved diagnostic sensitivity.

YS2-049-003

On farm monitoring of the four Q's of colostrum management is important.

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Objectives: Colostrum management is crucial to provide calves with passive transfer of immunity, as calves are born agammaglobulinemic. Failure to ingest sufficient quantities of good quality colostrum is a major risk factor leading to mortality and morbidity in neonatal calves in the UK.

The objective of the study was to assess the colostrum management on 6 commercial dairy farms in the UK. All four Q's (Quantity, Quickly, Quality, and sQueaky clean) were assessed and compared to target measures.

Materials and Methods: A total of 206 colostrum samples were collected from cows and heifers from six UK dairy herds in the Wiltshire and Gloucestershire area, between April and July 2013. Herds were recruited due to their interest in calf management. Both volume of colostrum, and the time colostrum was fed to calves were recorded. Each farm was asked to collect a sample of colostrum at the point which the colostrum entered the calf (e.g. out of the oesophageal feeding tube) into a sterile milk sample pot provided, and to label appropriately. Samples were stored between 4-8°C for a maximum of 48 hours before submission. At the practice laboratory, samples were assessed for quality by the same operator using a Brix refractometer, a reliable method of estimating IgG content. Samples were also cultured for 24 hours to count colony forming units (cfu) /ml, to determine the bacterial load.

Results: The mean volume of first colostrum given on farms was 3.6 litres, ranging from 2.0 – 4.0 litres. Only 57% (117/206) of calves received the target 4L of colostrum as an initial feed.

The mean calving- colostrum administration interval was 5.4 hours with considerable variation between farms, with farm 2 mean time being 3.4 hours compared to farm 5 being 6.7 hours. Since more samples were collected from farms 1 and 2, the majority of calves (57% 118/206) received colostrum within the target 4 hours of birth.

The mean value for colostrum quality, as measured by percentage Brix (%Brix), was 23% and ranged from 14-31%. Only 40% (82/206) colostrum samples had a %Brix value above the target 22%, which equates to 50g/dL IgG. There was a large variation both within and between farms. In samples where the source was known (n=80), there was a significant difference between heifer and cow colostrum, with heifers' mean value being 21.8% (SD 0.6), compared to 23.7% (SD 0.6) for cows (P=0.02 using a two-sample t-test with equal variance).

The mean bacterial count was 129,391cfu/ml. The target bacterial count is less than 50,000cfu/ml, and counts over 250,000cfu/ml have been linked to fatal enterotoxaemia. Only 17% (29/175) samples were below target (50,000cfu/ml) and 9% were above 250,000cfu/ml. There was considerable variation between farms as well as within farm results. Farm 1 had the highest mean bacterial count at 230,039cfu/ml, ranging from 98,000cfu/ml to 831,000cfu/ml. Farm 3 had the lowest mean bacterial count at 84,393cfu/ml ranging from 10,000 to 295,000cfu/ml.

Conclusions: Less than two thirds of calves received either adequate volume of colostrum or received it within adequate time frame. Other concerns are colostrum quality and bacterial load. Colostrum quality was below the target %Brix value in 40% of samples, indicating a low level of IgG. Very few farms currently monitor colostrum quality and then adjust volume fed accordingly. Measurement of colostrum cleanliness is not routinely undertaken in the UK. The high bacterial load in many colostrum samples in this study indicates colostrum cleanliness should be assessed as part of any investigation into colostrum management.

Comments: This study highlights the importance of including on-farm monitoring of colostrum management, and assessing all four Q's. As the farms selected had been chosen for their interest in calf management, it is possible that results for the national herd could be worse. Where targets are not being met, intervention measures can then be implemented to help reduce the risk of disease in neonatal calves.

YS2-049-004

Effects of dietary forage on rumen pH and bacterial community in Holstein calves during weaning transition.

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Objectives: Excessive intake of rapidly fermentable carbohydrate causes rumen acidosis, and disturbs the normal flora of rumen. Although several studies have attempted to investigate the effect of feeding material on rumen environment, the relevance between ruminal pH and microbiota during weaning transition still remains unclear. Therefore, the objective of this study was to investigate the relationship between ruminal pH and bacterial communities when feeding the calf starter with or without forage to dairy calves during weaning transition.

Materials and Methods: Sixteen rumen cannulated Holstein bull calves were raised using milk replacer (24% CP and 20% fat), calf starter (22% CP and 3% fat) and alfafa hay. Calves were randomly assigned into calves fed calf starter with forage (HAY, n=8) and only a calf starter (CON, n=8). Ruminal pH was measured continuously in every 10 min by radio transmission pH measurement system during the experiment. Ruminal fluid samples for VFA analysis and total bacterial DNA extraction were

collected from the ventral sac of the rumen, the morning prior to feeding at -1, 0, 1, and 3 weeks after the weaning. Ruminal bacterial composition and the copy number of 16S rRNA of bacterial species were analyzed by 454 pyrosequencing and quantitative real-time PCR, respectively.

Results: 24-h mean pH of rumen was affected by diet, and HAY group had higher ruminal pH when compared with CON group ($P < 0.001$). The ratio of ruminal acetate to propionate were significantly increased ($P < 0.05$), and a tendency towards increased ruminal propionate level was observed ($P = 0.0849$) in the CON group. There were significant differences between HAY and CON group in propionate level ($P < 0.05$) at 1 week and the ratio of ruminal acetate to propionate at 1 ($P < 0.01$) and 3 ($P < 0.01$) weeks. By the 454 pyrosequencing, HAY group presented significantly higher bacterial richness in Chao1 ($P < 0.05$) and ACE ($P < 0.05$), and Shannon index indicated that HAY group had a tendency ($P = 0.0529$) to have higher bacterial diversity than the CON group. When compared with CON group, there were significant differences in the relative abundances of phyla (Bacteroidetes; $P < 0.05$ and Actinobacteria; $P < 0.05$) and genus (Prevotella; $P < 0.05$) in HAY group. By the qPCR, HAY group was affected by diet in the copy number of *Ruminococcus albus* ($P < 0.05$) and *Ruminococcus flavefaciens* ($P = 0.0872$), and there were also significant changes in the copy number of total methanogen ($P < 0.05$) and *Megasphaera elsdenii* ($P < 0.01$) over time. When compared with CON group, the copy number of *R. flavefaciens* ($P < 0.05$) and *R. albus* ($P < 0.05$) were significantly increased at 1 and 3 weeks in HAY group, respectively.

Conclusions: Calves fed calf starter with forage had significantly higher 24-h mean ruminal pH when compared with calves fed only a calf starter. The relative abundances of Bacteroidetes, Actinobacteria, and Prevotella were significantly affected by diet and the copy number of 16S rRNA of cellulolytic bacteria, such as *R. albus* and *R. flavefaciens*, were significantly increased in HAY group. Therefore, forage and calf starter feeding to calves is related to ruminal pH and bacterial communities, and may affect the establishment of fermentative ruminal functions during weaning transition.

YS2-049-005

Prevalence, associated clinical conditions and prognostic relevance of hypoglycaemia in 7,000 hospitalized neonatal calves

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Objectives: Hypoglycaemia is known to occur as concomitant of neonatal diarrhoea and experimentally induced endotoxaemia in calves. However, the relevance of this metabolic imbalance in spontaneously diseased calves has been poorly investigated. The objectives of the present study were therefore to determine the prevalence of hypoglycaemia in a large population of hospitalized neonatal calves, to identify the underlying health problems that are associated with this condition, to assess the clinical predictability of hypoglycaemia and finally to analyse the possible impact on prognosis.

Materials and Methods: For the purpose of this study, the data of 7,000 neonatal calves (up to 21 days of age) admitted to the Clinic for Ruminants with Ambulatory and Herd Health Services, LMU Munich, between October 1997 and March 2008 were retrospectively analyzed. Clinical findings of a subset of 100 calves with severe hypoglycaemia

(plasma glucose concentration < 2 mmol/L) were extracted from the medical records and compared to those of 100 randomly selected calves with plasma glucose concentrations in the reference range (4.4 to 6.9 mmol/L). Plasma glucose concentrations were determined on admission to the hospital and survival was defined as discharge from the hospital.

Results: The prevalence of severe hypoglycaemia was 6.0%. In those calves the survival rate was 18.8%, which was poor opposed to 75.6% discharged animals in the group of calves with initial plasma glucose concentration in the reference range. The overall recovery rate was 66.8%. A binary logistic regression analysis indicated that in calves with a plasma glucose concentration of < 7 mmol/L the odds for non-survival increased by 57.5% for every mmol/L decrease of plasma glucose concentration.

Review of the medical records revealed that severe hypoglycaemia was significantly associated ($P < 0.001$) with clinical and/or post-mortem evidence of septicaemia, generalized peritonitis of varied origin, severe hypothermia and a history or clinical diagnosis of severe malnutrition. However, the proportion of calves with signs of central nervous involvement like seizures and opisthotonus was not significantly different between calves with severe hypoglycaemia and calves with normoglycaemia.

Conclusions: Hypoglycaemia in hospitalized neonatal calves is of rare occurrence, but is associated with serious health problems and therefore represents a reliable indicator of non-survival. We concluded that hypoglycaemia should be suspected in neonatal calves with an abdominal emergency, clinical evidence of septicaemia and a history or signs of malnutrition. However, a hypoglycaemic state can only be suspected clinically, indicating that low-cost, reliable and rapid on-farm test-methods would represent a helpful tool in critically ill neonatal calves.

POSTER PRESENTATIONS

Abortion/Stillbirths
Animal Health Economics
Animal Welfare
Anti-Microbial Resistance
BVD
Camelids
Clinical Genetics
Complementary Medicine
Diagnostic Imaging
Education
Emerging Diseases
Epidemiology
Genetics And Breeding
Herd Health Management Beef
Herd Health Management Dairy
IBR
Immunology/Vaccinology
Internal Medicine
Lameness
National Animal Health Programmes
Neosporosis
Nutrition And Metabolic Diseases
Parasitology
Paratuberculosis
Public Health
Reproduction
Reproduction (Beef)
Reproduction (Dairy)
Reproductive Technology
Salmonellosis
Small Ruminants
Surgery
Sustainable Agriculture
Therapeutics And Pharmacology
Toxicology
Tuberculosis
Udder Health And Milk Quality
Young Stock

Abortion/Stillbirth Investigation

P04-004-001

A New Obstetric Tool: Can GYNstick Really Make A Difference? - Survey Among Bovine Practitioners

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Objectives: Dystocia in cattle affects up to 50% of primiparous and 30% of adult cows¹. Obstetrics are crucial in order to reduce stillbirth rates. GYNstick^{2,3,4}, a new tool for treating dystocia, has been available for practitioners for almost 3 years. Nearly 400 vets in Germany and Austria are currently practising with this obstetrical instrument in their daily practice, and approximately 2000 vets are using it worldwide. A survey was conducted among 49 German and 5 Austrian practitioners to obtain feedback about their experience with this instrument and their recommendations for its use.

Materials and Methods: 54 veterinarians responded to an online questionnaire (Monkey Survey) initiated by the Faculty for Veterinary Medicine of the University of Giessen. Veterinarians were chosen based on their practical experience with this new instrument for at least 1 year. The data analysis (SPSS Version 22; IBM inc) compared the veterinarians experiences by gender, age and frequency of dystocia interventions. The participants have been identified by hazard out of a data sets of GYNstick distributors. 196 users have been invited to reply, 54 responded finally and completed the questionnaire (27,55 %).

Results: Most of the practitioners (64%) stated that GYNstick was recommended to them by another colleague. The geometrical mean of dystocia cases per week was 1.52 (female, 1.44 vs. male, 1.63; p=0.206; t-test). For 83 % of the responders, the correction of a twisted uterus was the most frequent indication for using the instrument prior to other indications like correction of malpositionings or reach of displaced extremities.

Most veterinarians (92.5 %) think that the instrument reduces the duration of dystocia, without differences between males and females (p=0.9, X²). Forty-three percent of the vets estimated a 30% time reduction. Forty-four percent estimated a 30 - 50% time reduction. Approximately 2/3 (65 %) of the vets felt that the instrument did not impact the number of live calves born. For the remaining veterinarians, 24% thought that number of calves born alive was 30% greater after implementing this tool. Nine percent of veterinarians estimated an increase in live calves of 50% (no gender differences; p=0.1, X²).

84% of the practitioners are convinced that the instrument is a more effective life-saver than traditional obstetrical instruments.

All users are convinced that it is less exhausting in comparison to other tools or manual interventions. In addition, 21% said that muscle efforts are reduced by 50-80%. Interestingly, this outcome was not impacted by sex of the veterinarian (p=0.1, X²).

All respondents replied that they would recommend (81% full agreement, 19%, recommend if asked) the tool to their colleagues.

Conclusions: A new obstetrical instrument, the GYNstick, was well accepted among veterinary practitioners in Germany and Austria. Most users were female and graduated in the last 14 years. All respondents would recommend it to their colleagues. Veterinarians should consider using this tool to reduce the physical impact of difficult calvings to newborn calves and their dams. And finally it makes life easier for vets as well - in a gender changing bovine vets world really an important and actual aspect.

Abortion/Stillbirth Investigation

P04-004-002

Prevalence of gross pathological lesions indicative of traumotocia in stillborn calves in Polish dairy herds

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Objectives: Dystocia is the most common cause of perinatal calf mortality. Disproportion between pelvic size and the size of the calf, fetal malposition and inappropriate calving assistance may lead to trauma and fetal death. Evidence of inappropriate calving assistance and associated trauma can be detected during post mortem examination. If inappropriate calving assistance resulting in trauma is the main cause of death this is defined as traumotocia.

Materials and Methods: The study was carried out on 121 stillborn (SB) calves born dead after a gestation period of ≥ 260 days or died within 6 hours of birth, from November 2013 to June 2015 in 30 Polish Holstein-Friesian herds (1-1,031cows/herd; 1-35 SB/herd). Data were recorded on calving assistance, fetal position and the time of death after birth for calves which were born alive. All calves were subjected to a complete laboratory necropsy within 12 hours after calving, and in the calves which were born alive, after death. Each carcass was examined by the same protocol. Lesions of trauma were recorded, categorized and their contribution in the cause of death was ranked. Calves with traumotocia were assigned to one of five mutually exclusive groups according to the extent/severity and type of trauma: (1) blood vessel rupture leading to extensive internal haemorrhage, (2) spine fracture with associated hematoma, (3) large ($\geq 10\text{cm}^2$) diaphragm tear, (4) multiple (≥ 3) ribs fractures with associated haematoma and (5) other causes.

Results: Of the 121 calvings (28 calves from twin calvings), 114 (94%) were observed [of which 111 (92%) were assisted and 3 were observed/unassisted] and seven (6%) were unobserved. Of the 111 assisted cases (75 with calving jack), 24 (22%, singleton calvings) had lesions associated with traumotocia. In six cases blood vessel rupture [vena cava caudalis (4 calves), vena cava cranialis (1 calf) and diaphragm vessels (1 calf)] was associated with extensive haemorrhage into the abdominal cavity (2 cases), thoracic cavity (3 cases), and both cavities (1 case). Spine fracture with associated haematoma was observed in three calves (Salter-Harris type I spine fracture at the thoraco-lumbar or L₁-L₂ junction). In two calves there was a large diaphragmatic tear. Fracture of the ribs with associated haemorrhage was the most commonly observed traumotocic pathology (12 cases). Such fractures were most commonly located at the costochondral junction (8 cases). In one case extensive occipital trauma including haemorrhage was detected (associated with a calf falling on its head on to a concrete floor). In addition to these lethal lesions, there were associated sublethal lesions of traumotocia in the majority of these calves.

Conclusions: This study showed that in these Polish dairy herds approximately one fifth of stillbirths were caused by traumotocia; half of these were caused by fractured ribs. These findings have implications for veterinary education of farm personnel in when to assist and how to use mechanical calving aids when assisting difficult calvings.

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Abortion/Stillbirth Investigation

P04-004-003

Diagnosis of Neospora and Leptospira in bovine abortions/stillbirths – how does foetal serology compare with tissue qPCR testing?

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Objectives: Neospora caninum and Leptospira Hardjo were detected in 4.2 and 7.4% of Irish bovine fetuses (2013), respectively. As diagnosis of both relies mainly on foetal serology, under-diagnosis is likely. In only approximately a third of abortion cases is a cause diagnosed. Recently, validated quantitative real time PCR tests have been developed for these foetopathogens (FarmLab Diagnostics). By combining foetal serology and qPCR it may be possible to address the issue of under-diagnosis. Hence, the objective of this study was to compare the infection detection rate of serology and molecular diagnostics in the same aborted/stillborn fetuses.

Materials and Methods: Foetal blood/transudate, kidney and brain samples were collected from 141 aborted fetuses and perinates (138 were tested for Neospora and 141 for Leptospira) in 36 dairy herds in the main dairying region of Ireland (Munster), (1-12 fetuses/herd) between January and June, 2015. None of the perinates had consumed colostrum. Neospora serology was conducted using an IgG ELISA (IDEXX Bovine Serum Neospora Caninum, IDEXX, Bern, Switzerland, (sensitivity of 95.8% and a specificity of 100%) with results reported as negative (<30% optical density, OD), inconclusive (30-40% OD) or positive (>40% OD). Leptospira serology was conducted using an ELISA (PrioCHECK L. hardjo Ab, Prionics, Zurich, Switzerland) which detected anti Leptospira hardjo IgG (sensitivity of 99% and specificity of 85%). The results are reported as negative (<40% OD), inconclusive (40-60% OD) or positive (>60% OD). Both tests were run in the Cork Regional Veterinary Laboratory. Following gDNA extraction of kidney (for Leptospira) and brain tissue (for Neospora), purified samples were tested by real-time PCR with the target-specific TaqMan primers. Both qPCRs were run in FarmLab Diagnostics Laboratory.

Results: In total 26 (18.8%) fetuses were either Neospora ELISA or qPCR test-positive; 8 (5.8%) and 18 (13%) Neospora ELISA and qPCR test-positive, respectively. Of 8 Neospora ELISA test-positives, 2 (25%) were Neospora qPCR test-positive. Of 18 Neospora qPCR test-positives, 2 (11.1%) were Neospora ELISA test-positive. In total, 2 (1.5%) fetuses were both Neospora ELISA and qPCR test-positive. Neospora ELISA and qPCR test-positives were detected in 6 (17.1%) and 11 (30.5%) herds, respectively. In total 26 (18.4%) fetuses were either Leptospira ELISA or qPCR test-positive; 0 and 26 (18.4%) Leptospira ELISA and qPCR test-positive, respectively. Of 0 Leptospira ELISA test-positives, 0 were Leptospira qPCR test-positive. Of 26 Leptospira qPCR test-positives, 0 were Leptospira ELISA test-positive. In total, 0 fetuses were both Leptospira ELISA and qPCR test-positive. Leptospira ELISA and qPCR test-positives were detected in 0 and 17 (47.2%) herds, respectively. Of 26 Leptospira qPCR test-positive fetuses, 2 (7.7%) were also Neospora qPCR test-positive and of 18 Neospora qPCR test-positive fetuses, 2 (11.1%) were also Leptospira qPCR test-positive. Both cases of co-infection were from the same herd.

Conclusions: It is concluded that these preliminary data indicate a much higher foetal infection detection rate with qPCR compared to foetal serology, particularly for pathogenic Leptospira. There was poor agreement between fetal serology results and qPCR results for both foetopathogens, in particular for pathogenic Leptospira. While it is accepted that foetal infection does not prove cause of death, the higher

detection rate of foetopathogens in these fetuses by qPCR warrants further investigation. It is recommended that more widespread sampling nationally be conducted before extrapolation of these results.

Abortion/Stillbirth Investigation

P04-004-005

Potential Nutritional Causes Of Stillbirth In Dairy And Beef Calves

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Objectives: Stillbirth remains a significant reproductive loss to the dairy and beef cattle industries. Dystocia accounts for a large proportion of stillbirths, but at least 30-40% of stillbirths have no definitive diagnosis. The study objective was to identify possible nutrition-related causes of stillbirth in dairy and beef calves.

Materials and Methods: In this study a stillbirth was defined as a calf between 265 days gestation and 6 hours postcalving. Stillborn calves meeting this definition were collected from various dairy and beef cattle farms throughout Pennsylvania. If historical evidence suggested the stillbirth was due to dystocia, then the submission was not included in the study. All eligible stillborn calves underwent a full diagnostic necropsy procedure (gross and histopathology) including measurements of body weight and crown-rump length measurement. Calf gender, haircoat condition, lung insufflation status and any obvious physical abnormalities were noted. Microbiologic evaluations specifically addressing BVD, IBR, leptospirosis and Neospora were completed on all submissions as well as routine microbiologic culture of various tissues. Liver samples were collected and submitted for mineral analysis including calcium, magnesium, cobalt, copper, iron, manganese, selenium and zinc using ICP/MS methodology. Measurement of vitamins A (total retinol) and E (α-tocopherol) concentration were by high pressure liquid chromatography (HPLC). All values were determined on a wet weight (WW) basis and converted to dry weight (DW) based on measured dry matter ratio of liver samples. A section of the thyroid gland was collected for histopathologic evaluation to assess iodine status. Analysis of variance (ANOVA) statistical methods were used to determine potential effects of breed (dairy or beef), gender, season or diagnostic category (infectious, congenital, idiopathic) on mineral and vitamin measures.

Results: To date a total of 27 calves (7 beef; 20 dairy) met the case definition and had all determinations completed. Beef calves were of various breeds, but predominately Angus and dairy calves were predominately Holstein breed. Mean crown-rump length and birth weight was 93.7 ± 9.3 cm and 31.0 ± 8.6 kg for dairy calves and 91.8 ± 8.2 cm and 28.2 ± 7.5 kg for beef calves, respectively. There was one large beef calf (55 kg) and one small dairy calf (16.4 kg) not included in these means. Overall gender distribution was equal, but beef calves were predominately female (71%) whereas dairy calves were more males (60%). No calf had inflated lungs with 69% and 31% with none or partial lung insufflation, respectively. Two (7.4%) calves were considered to have congenital defects, 7 (25.9%) calves had an identified infectious agent and 17 (63%) of cases had no definitive stillbirth diagnosis. Overall there were 1.6 (range: 0-4) mineral deficiencies and 0.8 (range: 0-2) vitamin deficiencies per calf. Total mineral and vitamin abnormalities or deficiencies per calf were 3.1 and 2.4, respectively. Vitamin A deficiency (<8 µg/g DW) was the primary (18/27, 67%) vitamin abnormality. Low hepatic mineral concentrations were seen with cobalt (13/27, 48.1%),

selenium (11/27, 40.7%), manganese (7/27, 25.9%), iron (5/27, 18.5%), copper (2/27, 7.4%) and zinc (3/27, 11.1%). Excessively high iron and zinc concentrations were found in 6 (22.2%) and 11 (40.7%) cases, respectively. Effects of gender, breed and crown-rump length did not influence mineral or vitamin concentrations.

Conclusions: These preliminary data strongly support the role of trace mineral and vitamin deficiencies as potential underlying causes of stillbirth losses in dairy and beef cattle herds. Further study is warranted to confirm these results and identify specific mechanisms possibly related to antioxidant status.

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Abortion/Stillbirth Investigation

P04-004-008

Laboratory findings suggesting an association between BoHV-4 and bovine abortions in southern Belgium

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Objectives: Abortions cause heavy economic losses for the bovine sector. The use of a standardized panel of analyses covering a large spectrum of pathogens responsible of abortion in cattle, allowed demonstrating the direct involvement of at least one pathogen in 57% of analysed abortions in the southern part of Belgium. This result suggests a margin of improvement in the diagnostic efficacy. In order to evaluate the interest to broaden the list of pathogens included in the panel of analyses, the implication of bovine herpesvirus 4 (BoHV-4) in abortion was assessed by two different studies.

Materials and Methods: Two studies were conducted in the southern part of Belgium, including five different provinces (Walloon Brabant, Hainaut, Namur, Liege and Luxembourg). In the study area, 12,000 cattle herds with 1,257,000 bovines are registered. In the first study, coupled serology was performed after abortion on 714 dams to identify specific seroconversion against BoHV-4. In the second study, the virus ability to infect the foetus was investigated and 368 cases of bovine abortions were specifically tested for BoHV-4, with PCR on foetus tissues and ELISA on dam and foetus sera.

The diagnosis of BoHV-4 in the maternal serum was performed using an indirect ELISA test (Edwards and Newman, 1985; Wellenberg et al., 1999) according to the manufacturer's instructions (BIO K 263/2 ©, BioX Diagnostics, Rochefort, Belgium). Concerning the foetal serology, the same ELISA was performed using a 6 mm disc of saturated blood (Bailey et al., 1967).

Direct diagnosis of BoHV-4 was performed on spleen fragments stored at -20 °C. DNA extraction was performed using KingFisher TM Flex 96 Magnetic Particle Processors (Thermo Scientific TM, UK) and LSI MagVet TM Universal Isolation Kit (Life Technologies, UK) and was followed by BoHV-4 DNA detection using a commercial PCR test (LSI VetMax BoHV-4 © TM, Life Technologies, UK), according to the manufacturer's instructions (Wellenberg et al., 2001). The PCR reactions were performed with a Stratagene Mx3500P (Agilent Technologies, USA). According to the manufacturer's instructions, a sample was considered positive for a threshold cycle (= Ct) value lower than 46.

Results: During the first study, 760 cows out of 1,158 (65.6%; 95% CI: 62.8-68.4%) were seropositive at first sampling. 714 cows were sampled a second time after abortion for a coupled BoHV-4 serology. Based on an increase of at least two orders of magnitude of the S/P ratio between the first and the second sampling, 113 cows were seropositive at the coupled serology against BoHV-4, among the 456 cattle that were seropositive only at first sampling. Out of 258 seronegative cows at the time of abortion, 26 seroconverted at the second sampling, suggesting a correlation with BoHV-4 primary infection.

During this second study, antibodies against BoHV-4 were identified in sera of 279 cows out of 368 cows collected after abortion (75.8%; 95% CI: 71.1-80.1%). The DNA of BoHV-4 was detected by PCR in the spleen of four fetuses (1.1%) and for three of them, no other specific pathogens or opportunistic bacteria could be identified. These four fetuses aged between 6 and 9 months and came from seropositive cows at the time of abortion. However, these four fetuses were all seronegative regarding to BoHV-4. On the contrary, in the foetus where the highest Ct value for BoHV-4 could be measured in the spleen, an opportunistic bacterium (*Trueperella pyogenes*) was isolated from the abomasal fluid. The four PCR-positive samples presented a Ct value varying from 23 to 36. Three BoHV-4 seropositive but PCR-negative fetuses (0.8%) were detected and were derived from seropositive cows. Opportunistic bacteria were isolated on the abomasal fluid of one of these three cases (*E. coli*).

Conclusions: Despite the fact that the role of BoHV-4 as etiologic agent of a specific entity remains a matter of controversy, these two studies showed a high seroconversion for this pathogen in bovine at abortion, the proof of a foetal immunity response and the direct diagnosis of this virus in foetal spleen. These results constitute additional arguments of the possible involvement of BoHV-4 in abortion in the southern part of Belgium.

Abortion/Stillbirth Investigation

P04-004-009

Genomic-based identification of host and environmental *Listeria monocytogenes* strains associated with an abortion outbreak in beef heifers in the US

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Objectives: *Listeria monocytogenes* (LM) can cause late-term abortions in cattle. In a listerial abortion outbreak, objectives for this diagnostic work-up were to employ novel case and environmental sample collection protocols, then define the probable cause of the outbreak by comparing LM strains isolated from cases and their environment through whole-genome sequencing.

Materials and Methods: Twenty crossbred heifers that aborted from late January into February, 2014, were identified. Presumptive diagnosis of listerial abortion was based on two LM culture positive, late-term aborted fetuses. Proximal vaginal/distal cervical swabs (VCS) or retained fetal membranes (RFM) from heifers were then submitted for LM culture and PCR. Silage sampled via a grid system protocol and water sources were also submitted for culture and PCR. Resulting strains were subjected to whole-genome sequencing and compared.

Results: Heifer submissions identified 16/20 LM positive samples; 10/10 RFM and 6/10 VCS were positive, respectively. 11/45 environmental

samples were positive. Because definitive diagnosis and point-source determination can be challenging, genomic sequencing of case and environmental isolates was performed, demonstrating that 10/18 fetal/heifer samples and all environmental samples were a related strain. Remaining case strains were similar to each other, but genetically distinct from all other case and environmental strains.

Conclusions: When fetal materials are unavailable, VCS and RFM samples are an effective means to diagnose abortions caused by LM. Sampling feed in an organized, documented manner may help identify the source. In this investigation, two LM case strains were identified, one genetically linked to contaminated silage and/or water, while the source of the other strain remains unknown.

Abortion/Stillbirth Investigation

P04-004-010

Veterinarians and farmers' perceptions of abortion in cattle and their barriers and motivators towards abortion investigations

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Objectives: Abortion in cattle reduces production efficiency and causes significant economic loss to dairy and beef farmers. Abortion investigations are pursued in variable frequency by veterinarians and their clients. This may be due to a lack of information or because the diagnostic success rate is poor; even when a foetus and placenta are submitted, the reported diagnostic rate in the UK is still estimated to be 35%.

Materials and Methods: The aim of this study was to gain an understanding of veterinarians' perceptions of cattle abortion on their clients' farms and to determine their barriers and motivators towards abortion investigations. The veterinarians' perceptions were then compared to farmers' perceptions towards cattle abortion investigations, based on data collected in a previous study.

A questionnaire-based survey of 134 British and Irish veterinarians collected information on the perceptions of cattle abortions and determined barriers and motivators of the veterinarians, as well as their perceptions of farmers' barriers and motivators, towards abortion investigations. Results were compared to farmers' barriers and motivators towards cattle abortion investigations, which were collected previously in a similar questionnaire surveying 379 British and Irish cattle farmers.

Results: Veterinarians and farmers had similar perceptions of the definition and estimated incidence of cattle abortion. Veterinarians were mainly deterred from recommending sample submission by lack of good quality samples, low diagnostic rate and cost. Veterinarians and farmers were receptive to help to reduce cattle abortion and many requested an improved laboratory service. The majority of respondents were motivated to recommend sample submission in the presence of a high abortion incidence and felt an incidence higher than 4% warranted investigation. Veterinary respondents overestimated cost and underestimated lack of concern or understanding as barriers for farmers to investigate abortions. Overall, it seemed veterinarians did not expect clients to be prepared to pay as much as farmers were willing to pay for an abortion investigation; three times as many farmers as veterinarians felt that they should pay € 501-1000 for all applicable laboratory analyses. Veterinarians also overestimated cost as a barrier and reduced cost as a motivator to farmers regarding the submission of samples for abortion investigation. Nineteen per cent of veterinary respondents

were deterred from recommending farmers to submit samples due to the cost of investigation. There was a disparity between the percentage of veterinarians recommending abortion investigation (97%) and farmers who actually indicated to submit samples for investigation (51%). Improved communication between veterinarians and farmers may increase uptake of abortion investigations.

Conclusions: The study gained an insight into British and Irish veterinarians' barriers and motivators to, and perceptions of, cattle abortion investigation, as well as their perceptions of farmers' attitudes. Addressing barriers to abortion investigations in cattle, such as improving knowledge transfer and the practicalities of submitting samples, as well as differences between farmers' attitudes and veterinarians' perceptions of farmers' attitudes, may result in improved uptake of abortion investigation services.

Abortion/Stillbirth Investigation

P04-004-011

Can veterinary practitioners guesstimate how long a foetus is dead in utero using corneal opacification as a guideline?

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Objectives: Once somatic mortality occurs post-mortem decomposition begins. The rate at which this occurs is dependent upon the cause of death, environmental and body temperature and microbial flora. For foetuses which die in utero it is usually not possible, under field conditions, to know when they died. One indicator which may be of value in estimating the time of death relative to time of birth (intrauterine retention time) is corneal opacity (clouding, blueing). To test this hypothesis a pilot study was conducted using newborn calves.

Materials and Methods: Four eyes from four Holstein-Friesian newborn calves with known time of death were used. Two eyes were enucleated and placed in plastic bags in a water bath at 38.5° for 96 hours (4 days). This was to represent a dead in utero simulation (DIU). The other two eyes were left in situ in the skull and placed in a pen at ambient temperature (January & March), for 168 hours (7 days). This was a control group (C). The gross changes in the eyes were recorded and photo-documented every 12 hours for the DIU and every 24 hours for the C eyes.

Results: The first signs of corneal opacity were visible at 24 and 48 hours in the DIU and C eyes, respectively. The degree of corneal opacity was substantially greater in the DIU compared to the C eyes at 24 and 48 hours, respectively. In addition peri-orbital tissue also showed signs of autolysis in the DIU group at 24 hours but not in the C eyes. Complete corneal opacity was visible at 36 and 96 hours in the DIU and C eyes, respectively. Again the degree of corneal opacification was greater in the DIU than in the C eyes at 36 and 96 hours, respectively. After 48 hours the turgor of the DIU eyes decreased but not in the C eyes. These findings suggest the onset of opacity in DIU eyes is between 12 and 24 hours, considerably earlier than at ambient temperature.

Conclusions: Corneal opacity may be used by veterinary practitioners as an indicator, along with others, of intrauterine retention time after bovine foetal mortality. Pre-partum opacity is not likely to be confused with peri or postpartum opacity in fresh carcasses which are examined within 24 hours of death. However, storing fresh carcasses at 4°C accelerates the onset of corneal opacity (and lens opacity). These results need to be repeated on a larger number of specimens and under varying simulation conditions.

Animal Health Economics

P04-004-012

Failure of passive immune transfer in calves: total cost and resources to be allocated for its managementDidier Raboisson¹, Clélia Cahuzac², Pauline Trillat²¹INP-ENVT INRA-IHAP, ²Toulouse Veterinary School, Toulouse, France

Objectives: Colostrum intake is reported as a key factor for the newborn calf health. Failure of passive immune transfer (FIT) is linked to no or low colostrum intake and is defined as low plasma IgG or total proteins at 24 - 48 hours of age. FIT is associated with an increased risk of mortality and morbidity. In spite FIT stakes are consensual, no economic assessment has been performed up to now. This work aims to (i) estimate the total cost of FIT, and to (ii) define the resources to be allocated to FIT, in the French context.

Materials and Methods: The total cost of FIT was calculated thanks to a stochastic two-step economic method. A meta-analysis (package Metafor, R) was firstly performed to clarify the adjusted links between FIT and morbidity or mortality. A dataset was drawn with the models of peer-reviewed papers reporting the odd-ratios (OR) of mortality or morbidity for calves with FIT. The co-variables of models and the various thresholds of IgG or total proteins used to define FIT were included in the dataset and in the meta-regressions. The economic model was secondly run, considering costs of extra diseases and mortality in case of FIT. All input parameters were laws of distribution and not point estimates. The model was run using Scilab with 10,000 iterations, and 95% prediction intervals (PIs) were calculated.

To define the resources to be allocated for FIT control, the net value at the farm level was calculated and the optimal economic situation was defined as the maximal net value. Technical indicators corresponding to the economic optimum can then be defined. The net value included (i) the production linked to calves, (ii) the cost of FIT for the given herd and for FIT prevalence and (iii) the cost of FIT prevention, which was estimated through time allocated to colostrum feeding. The relationship between time spent to feed colostrum and the prevalence of FIT was established thanks to literature. Cost of labor was established according to various scenarios so as to fit the field situations.

Results: The metaanalysis standardized the OR for mortality and morbidity by a unique threshold of total proteins (<50 g/L) or IgG (<10 g/L) to define FIT. The OR [and 95% confidence interval] of mortality, respiratory diseases and diarrhea were 2.12 [1.4-3.1], 1.75 [1.5-2.0] and 1.51 [1.05-2.17] for calves with FIT compared to no FIT, respectively. Calves with FIT has a 53 g. lower average daily gain compared to calves without FIT. These 4 components were included in the total cost of FIT.

The total cost [and 95 % PIs] of FIT was 60 euros [10-140] and 80 euros [20-110] per calf with FIT, for dairy and beef calves, respectively. Mortality, respiratory diseases, diarrhea and average daily gain contributed for 26, 16, 13 and 45 % of the total cost. The total cost of one case can be easily used in the field to calculate the avoidable costs of FIT for a given farm, thanks to comparison of the initial prevalence and the expected prevalence after changes in practices, multiplied by the unit total cost.

The optimal time to feed colostrum was around 15 min per calf, whatever the total cost of labor. Resources to be allocated for FIT management did not show important changes between scenarios. Importantly, increasing this duration led to small decreased in the farm net value, but decreasing this duration led to hugh decrease in the farm net value. Only very high cost of labor led to changes in the optimal time to be spent to feed colostrum. The optimal mortality and prevalence of respiratory diseases and diarrhea calculated at the economic optimum were 5, 29 and 23 % for most of the scenarios.

Conclusions: The total cost of one case of FIT is of interest to help decision making at farm level. Within few minutes, the practitioner can perform a raw estimation of the total cost. To prevent any error in decision making, both the mean cost and the ranges of the prediction interval have to be considered within the economic reasoning at farm level.

The present results also demonstrate the economic justification to spend at least 15 min per calf to make colostrum be ingested.

Animal Health Economics

P04-004-013

Effects of acute clinical infection with the Schmallenberg virus on milk yield, fertility and veterinary costs in Swiss dairy herdsMireille Meylan¹, Marianne Wüthrich¹, Isabel Lechner², Marlis Aebi¹, Andrea Vöggtlin³, Horst Posthaus⁴, Getraud Schüpbach-Regula²¹Clinic for Ruminants, ²Veterinary Public Health Institute, ³Institute of Virology and Immunology, ⁴Institute for Animal Pathology, Vetsuisse Faculty, University of Berne, Berne, Switzerland

Objectives: Schmallenberg virus (SBV) was first detected in Switzerland in July 2012 and many Swiss dairy farmers reported acute clinical signs in dairy cattle during the spread of the virus until December 2012. The objectives of the present study were to investigate the effects of an acute infection with SBV on milk yield, fertility and veterinary costs in dairy farms with clinical signs of SBV infection (case farms) and to compare them to a matched control group of dairy farms in which cattle did not show clinical signs of SBV infection.

Materials and Methods: Case and control farms were visited once between May and December 2013, and a questionnaire was filled with the herd managers. Data for cost of treatment and selected fertility parameters were retrieved from the veterinary bills, and data on milk yield and fertility were extracted from reports of the breeding associations. Data from 77 case farms, 84 control farms, and 77 matched pairs were available for analysis of variables from the questionnaire. Seventy-three case farms, 78 control farms, and 70 pairs had complete data for the analysis of veterinary costs and fertility parameters obtained from the veterinary bills (dystocia requiring veterinary assistance, retained fetal membranes, intrauterine treatments). Analyses of data from the breeding associations could be performed with 70 cases, 72 controls, and 63 pairs; 53 control and 52 case farms as well as 47 pairs were included in the analysis of calving intervals. In the statistical analyses, two different comparisons were made. Case farms were compared to control farms, and the time periods before, during and after SBV infection were compared within case and control farms, respectively. Data analysis was performed with univariable analysis followed by multivariable analysis in mixed regression models.

Results: Herd size was significantly ($p < 0.001$) higher in case farms (33 cows, $n = 77$) than in control farms (25 cows, $n = 84$). Within case herds, 14.8% (median) of the cows showed acute clinical signs. Managers from case farms indicated to have observed a higher abortion rate during the year with SBV (6.5%) than in the previous year (3.7%). Other fertility parameters showed no significant differences between the two groups. The general veterinary costs per cow from July to December 2012 were significantly higher ($p = 0.02$) in case (CHF 19.80; EUR 16.50) than in control farms (CHF 15.90; EUR 13.25). No differences in milk yield were found between groups, but there was a significant decrease in milk production in case farms in the second half year in 2012 compared to the

same period in 2011 ($p < 0.001$) and 2013 ($p = 0.009$). The average daily milk yield per cow (both groups together) was +0.73 kg higher ($p = 0.03$) in the second half year 2011 and +0.52 kg ($p = 0.12$) in the second half year 2013 compared to the same half year 2012. Fifty-seven percent of the cows with acute clinical signs ($n = 461$) were treated by a veterinarian.

The average calculated loss after SBV infection for a standardized farm was CHF 1606 (EUR 1338), which can be considered as low at the national level, but the losses were subject to great fluctuations between farms, so that individual farms could have very high losses ($> CHF 10'000$, EUR 8333).

Conclusions: In general, acute SBV infection in dairy cows had a short effect on animal health, but several farmers reported a long-lasting reduction in milk production in clinically affected animals. Overall within-herd prevalence of animals showing acute clinical disease was rather low, but multiple farmers observed high rates of clinically affected cows within their herds, which could lead to important economic losses in individual affected farms. Nonetheless, the economic impact of SBV infection appeared to be moderate at the national level.

Animal Health Economics

P04-004-014

Economic analysis of reproductive ultrasound examinations in a large-scale dairy herd – a field study

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Objectives: The reproductive performance of the Hungarian Holstein-Friesian cow population is relatively poor compared to other ICAR member countries. Transrectal ultrasound is a valuable tool in the reproductive management of dairy cows, however, it is still not used in a large proportion of the Hungarian dairies. Therefore, the aim of our study was to quantify the impact of the reproductive ultrasound examinations on the production parameters and the profitability compared to palpation per rectum in a large-scale dairy herd.

Materials and Methods: A field trial was carried out in a 420-cow Hungarian dairy herd between 6 October 2014 and 19 February 2015. The average 305-day milk yield was 8,009 kg in 2014. After checking the uterine involution on Day 42 postpartum, healthy cows were randomly assigned into two groups. In the palpation group ($n = 30$), all reproductive examinations – including the pregnancy diagnosis 40-47 days after insemination – were performed by rectal palpation, exclusively. In the ultrasound group ($n = 32$), all reproductive checks – and the pregnancy check 30-37 days after insemination – were performed via transrectal ultrasound examination. Reproductive examinations were carried out twice per week including pregnancy checks once a week.

In the economic analysis of the application of ultrasound examinations the cost of semen, the reproductive treatment costs (weighted average cost by active ingredients) and the veterinary fee were taken into account as costs, and the reduction of losses due to days open was considered as benefit. The average cost of an open day was quantified as the margin in net milk receipts (income over feed cost) and net income from calf sales between cows with the optimal and the actual calving interval. The average costs and prices of the study period were used in the calculations, where 1 EUR = 300 HUF (milk price: 36.67 EUR cent/kg, cost of ultrasound examination: 1 EUR/examination, semen cost:

11.67 EUR/straw, cloprostenol: 90.66 EUR cent/treatment, GnRH: 1.00 EUR/treatment, GnRH [cyst]: 2.00 EUR/treatment, cefapirin: 5.67 EUR/treatment, ceftiofur: 16.66 EUR/treatment).

Results: In the ultrasound group, the proportion of pregnant cows was 5.5% higher (68.8% vs. 63.3%), and these cows received less reproductive treatments (2.9 vs. 3.9 per cow) compared to the palpation group during the experiment. Concerning the pregnant cows in the ultrasound group, days to first service and days open were reduced by 7 and 12 days (74 vs. 81 days and 106 vs. 118 days), respectively, breeding interval was 29.6 days shorter (32.0 vs. 61.6 days), and these cows required 0.4 more inseminations (2.0 vs. 1.6 per cow) to conceive compared to the pregnant cows in the palpation group. Concerning the cows remaining open in the ultrasound group, days to first service was reduced by one day (71 vs. 72 days), and these cows received 0.6 more reproductive treatments (5.6 vs. 5.0 per cow) and slightly more inseminations (2.2 vs. 2.1 per cow).

The cost of an open day accounted for 6.5 EUR on average. The average cost of a reproductive treatment was 1.24 EUR in the palpation group and 1.40 EUR in the ultrasound group. Ultrasound examinations resulted in 73.1 EUR profit (benefit - cost) per pregnant cow compared to rectal palpation. Taking all the cows into consideration, ultrasound examinations caused 4.16 EUR extra cost per cow, however, the income increased by 53.62 EUR per cow on average. Therefore, earlier conception achieved by ultrasound reproductive examinations generated 49.46 EUR profit per cow compared to the palpation per rectum method, which means about 20,000 EUR annual extra profit in this herd.

Conclusions: Reproductive ultrasound examinations enable earlier reproductive treatments and inseminations, thereby reducing days to first insemination, days open and breeding interval, and resulting in more pregnant cows within the same time frame. Despite the additional costs, ultrasound examinations yielded a remarkable amount of profit compared to palpation per rectum in this herd. Hence, herd managers should consider applying reproductive ultrasound examinations in order to improve herd reproductive performance and profitability.

Animal Health Economics

P04-004-015

A Comparison Of The Costs Of Bluetongue Virus Serotype 8 (Btv-8) Surveillance And Intervention Programmes In Austria And Switzerland Between 2005-2013

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Objectives: The aim of this study was to assess the temporal and spatial distribution of costs incurred during the surveillance and intervention programmes to control BTV-8 in two comparable Alpine countries. Of particular interest was the proportion of costs covered by “in-kind contributions” made by the veterinary authorities and those which were reimbursed by the European Union. Finally, an assessment was made as to whether these costs are predictable and whether such predictions can be made accurately.

Materials and Methods: The total costs of the surveillance and intervention programmes in both countries were compared, with respect to “pre-outbreak”, “outbreak” and “post-outbreak” periods. The first case of BTV-8 in Switzerland was reported in 2007; in Austria in 2008. Costs were also calculated with respect to livestock units (LU). In Austria, 1 LU is defined as 500kg liveweight: approximately 1 adult cow or 6.7 adult sheep/goats. For the prediction of costs, a linear regression model was used. This model enabled the effect of animal species (cattle, sheep, goats) and the average farm size on total costs per political district to be calculated.

Results: Between 2005 and 2013, the total costs for the surveillance and intervention programmes in Austria amounted to €22.8 million, compared to €16.6 million in Switzerland. In Austria, 32% of these costs were incurred through surveillance and 68% was spent on intervention; whereas in Switzerland 94% of all costs were spent on the vaccination programme. Total vaccination costs were equivalent in both countries with around €15.5 million spent: in Austria these costs were primarily due to personnel expenditure, whereas the Swiss authorities spent almost equal proportions on personnel and material costs. The costs determined in this study for Austria were equivalent to €4.50/LU spent on surveillance and €9.64/LU spent on vaccination.

Of the total programme costs, “in-kind contributions” (e.g. blood samples taken by vets as part of their daily work) were estimated to amount to €2.1 million in Austria and just €229,681 in Switzerland. However, in-kind contributions are notoriously difficult to define and for this reason these costs are likely to have been up to 10-fold higher. The European Union reimbursed the Austrian state €4.9 million for costs incurred in enacting EU regulation 1266/2007.

The linear regression model demonstrated a good level of predictability for intervention costs in both countries: $R^2=0.97$ in Austria; $R^2=0.98$ in Switzerland. Given the large number of diagnostic tests available and differences in veterinary costs according to farm altitude etc., the model for predicting surveillance costs was found to be less accurate ($R^2=0.45$ in Austria; $R^2=0.28$ in Switzerland).

Conclusions: Despite Austria and Switzerland being comparable in terms of ruminant stocking density, landscape and climate, this study demonstrated that substantial differences existed with respect to costs incurred by BTV-8 surveillance and intervention programmes. It is perhaps also important to note that, between 2005-2013, a total of 76 farms were infected with BTV-8 in Switzerland, and 14 farms were infected in Austria over the same period. Our study demonstrates that variations in planning, implementation, personnel, material and overall strategy lead to large variations in costs between control programmes in neighbouring countries.

Animal Health Economics

P04-004-016

Internal economic losses due to culled and diseased dairy cows

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Objectives: Dairy cows differ considerably in their ability to adapt to their living conditions and to avoid the development of diseases and the risk of being culled. The operational management has direct impact on the portion of diseased and culled animals in the herd. Besides general

knowledge about economic impacts of production diseases, it is of high importance for the farmer to consider the degree of economic losses in the farm specific context, particularly the losses that occur in the case of diseased or culled cows in comparison to those animals that keep healthy. The objective of the study was to provide this information to the farm management.

Materials and Methods: The missing link between key economic figures and production data is seen as a significant weak point when trying to assess cost-benefit ratios in the context of production diseases. A new approach has been developed that enables an economic assessment of the consequences of increased rates of morbidity and culling as well as productivity losses, based on the production data of individual animals deriving from monthly available milk recordings. Data analysis is relying on a newly developed tool relying on the herd management software Herde® and based on individual lactation curves within a defined period of 365 days. Data transformation of culled cows and those that are persistent in the 365-day dimension is conducted separately for each lactation number. The average lactation curve progressions are linked with indicators of fertility (such as period between calving and first service, days open, pregnancy, etc.) and with the farm specific economic figures of production costs and revenues, reported in the agricultural production branch ‘cattle’ (BZA-cattle), including the realized milk revenue and sales of breeding animals and costs for replacement by heifers. The keeping costs include all costs that an average stock cow causes each day, based on the full-cost accounting. Also the revenue of slaughtered cows is included in the calculations. To assess the meaningfulness of the results deriving from this approach, 30 commercial dairy farms in Germany were selected in relation to general characteristics, resources, and management procedures.

Results: Data calculations revealed a large variation in productivity and economic losses between and within dairy herds. The variation resulted in considerable differences in the economic impacts of culled cows in relation to persistent cows regarding economic losses, and in the return of investment. The approach enables an action-related allocation of figures to assess the efficiency of production processes and the contribution of defined animal groups (e.g. healthy, diseased or culled cows) to the operating results. Moreover, robust assessments of the potential impact of changes concerning the economy can be made through an in-house comparison between defined groups of animals. Assessments of the accuracy of parameters provided good results, however, depending, on herd size. The profitability-analysis enables to quantify the impacts of variable factors (e.g. milk price, feed costs, culling rates, calving interval, prevalence of production diseases) and their changes over time on the development of livestock with its achievements and its economy. It facilitates the estimation of the contribution of different sub-groups to profit and losses, respectively, in relation to the overall economic results of the dairy production, providing decisive criteria for evaluating the effectiveness of operation.

Conclusions: Cost-benefit calculations are crucial to identify the most efficient measures and to show where investigations in animal health management may result in monetary benefits that exceed the investment. The new farm centric approach provides insights into the cost structure of a farm that have not been accessible before, thereby creating awareness and economic incentives for an improved animal health management.

Animal Health Economics

P04-004-017

Socioeconomic Impacts Of Foot-And-Mouth Disease On Smallholder Farmers In South-East Asia

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Objectives: In the global PCP (Progressive Control Pathway) for FMD, Pool I containing Southeastern and East Asia, is the second most advanced region involved in FMD control, with eradication achieved from Indonesia in 1984 and the Philippines in 2011, and a current OIE Roadmap encouraging regional FMD control through the SEACFMD program. To guide allocation of investments in FMD control, we conducted research on the socioeconomic impacts of FMD on large ruminant smallholders in Laos in 2011-12, including studies of gender, household financial status and farmer husbandry practices, including options and costs for treatment of FMD-affected animals.

Materials and Methods: Household level impacts of FMD were assessed by separate surveys (n=124, n=310) enabling stratification of wealth status as 'poor, medium or well-off' and FMD-attributable losses included those from mortality, morbidity and costs of treatments. National level FMD impacts were determined from reports of 2011-2013 FMD outbreaks and estimates based on: (i) total FMD financial losses at the village level; and (ii) the costs of FMD responses and other related costs but excluding the costs of revenue forgone. A Monte Carlo simulation accounted for scenarios of over- and under-reporting of FMD and above data used to estimate the benefit to cost ratio of the current vaccination program.

Results: Losses were USD436 (±92) in 'poor' and USD949 (±76) in 'well off' household categories (P < 0.001), being 128% and 49% of income from the sale of large ruminants, respectively. Variation in financial losses reflected differences in morbidity, farmer husbandry practices and importantly, choice of treatments, with adverse financial impacts where antibiotics were used. Monte Carlo simulation estimated that national financial losses in 2011 may have exceeded USD 102m, almost 12% of the estimated farm gate value of the national herd. Partial budget analysis of biannual FMD vaccination indicated an average net benefit of USD 22 and USD 33 for cattle and buffalo, respectively. Benefit to cost ratio of the current FMD vaccination program in northern Laos was estimated at 5.3.

Conclusions: FMD caused substantial financial impacts on households, villages and the national economy of Laos, with most severe impacts on poorer households, particularly as the inappropriate use of antibiotics for FMD created a financial burden. Females had a significant role in large ruminant production and financial management and extension programs that promote household investments in FMD control are financially justifiable and should involve women and importantly, avoid the use of antibiotics.

Comments: These findings support continuation of investments by international donors in FMD control in this region, including vaccination and biosecurity education. Socioeconomic perspectives are important where resources are severely limited and household and national expenditure on a relatively low mortality livestock disease that is of enormous global significance can be difficult to justify in developing countries, particularly when inappropriate animal treatment options are applied and unfortunately advocated by authorities.

Animal Health Economics

P04-004-018

Decision Support Tool For Implementing Vaccination Against Mastitis

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Objectives: Recent studies have documented the efficacy of STARTVAC against *S. aureus* (Schukken et al. 2014) and *E. coli* (Bradley et al. 2014). The former study stressed that the economic impact of STARTVAC in a specific herd depends on the herd's infection level with *S. aureus* and management level for udder health, such as milking procedures and culling regime of infected cows. Bradley et al. (2014) reported a return on investment of 2.6:1, but in the article it is stressed that this concerns the effects on milk yield alone and not the effect of the vaccine on the reduced severity of clinical cases.

Materials and Methods: SimHerd is a stochastic, mechanistic and dynamic simulation model of a dairy herd including young stock (Østergaard et al. 2005). The web-based model is routinely used by Danish veterinary practitioners and farm advisors and it quantifies the economic benefits of improving e.g. reproductive performance. In a collaboration between SimHerd Inc. and HIPRA, a decision support module was added to the existing SimHerd model that can estimate the economic return of using STARTVAC in a specific herd. The empirical evidence of the aforementioned studies was incorporated in the SimHerd model. Furthermore, SimHerd simulated the efficacy of the vaccine against *S. aureus* using the herd's infection level of *S. aureus* as herd specific data, but also for four different management levels for udder health. The economic impact of the vaccine against *E. coli* was estimated by simulating a scenario in which the vaccine's effect on milk yield alone was simulated and a scenario in which the effect on milk yield and reduced severity of mastitis cases was simulated. The herd's level of mastitis incidence and distribution of severities was entered into the model as herd specific data. Other herd specific data that was used in the SimHerd model, was among others milk yield level, reproductive performance, replacement rate and other diseases treatments.

Results: The simulated results of the four *S. aureus* scenarios and two *E. coli* scenarios are summarized in a three-page report in pdf. The report contains bar diagrams showing changes in net return per cow per year for the different scenarios, in contrast to the costs of the vaccine, represented by a horizontal line in the bar chart. The report furthermore presents development of economic return over time and technical details for each scenario, such as changes in milk yield per cow per year and culling rate. The simulated increase in net return (excluding costs of vaccination) when using STARTVAC in a typical 200-cow Danish dairy herd is €3087 and €13825 per year for the efficacy against *S. aureus*, in case the management level is poor and good, respectively. The increase in net return with respect to the efficacy against *E. coli* is €7919 and €14630 when simulating the effects of milk yield alone and the effects of both milk yield and reduced severity of clinical cases, respectively.

Conclusions: SimHerd with the additional module for estimating the economic impact of STARTVAC has since the development been used by Danish veterinarians. The simulated results of the vaccine efficacy are used for decision support but also in the valuable discussion between farmer and veterinarian on management practices of udder health. Simherd has already supported decisions to vaccinate herds in Denmark.

Animal Health Economics

P04-004-019

Evaluating Periparturient Disease Costs in Dairy Cows Using the Economic Assessment Tool

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Objectives: The Vital 90™ Days span from 60 d prior to calving through 30 d in lactation. During this time, dairy cows experience negative energy balance and immune suppression as well as metabolic and infectious problems. Cows that experience these issues often have higher culling and mortality risks; survivors often experience diminished milk production and reproduction. The objective of this project was to examine the total cost associated with transition dairy cows, including investment and consequence costs, via use of the Economic Assessment Tool and to understand factors that impact these costs.

Materials and Methods: The Economic Assessment Tool was created to understand the total costs incurred by dairy cows from dry off through the first 30 days of the subsequent lactation. After selection of the specific preventive procedures used in each time period by parity group (heifers vs. cows), general herd parameters such as milk price, feed price, culling risk, number of cows calving (by parity), fresh cow value, and market price for cull cows are entered. Fresh cow disease incidence is entered by parity group and the user specifies the typical treatment approach used for each disease including dose, duration, and milk withdrawal times. The model calculates the direct costs (diagnostics, therapeutics, discarded milk, labor, and death losses) and indirect costs (future milk losses, culling losses, and the cost of other attributable diseases). By summing the total direct and indirect disease costs, the total consequence cost is determined. Finally, the tool adds the total investment cost to the total consequence cost and reports The Vital 90 Days cost per cow calving by parity. Multivariate regression using the Fit Model platform and Standard Least Squares within JMP 12.1.0 software was used to examine the general management factors associated with herd-level disease consequence cost and as well as cow-level disease costs including ketosis and mastitis.

Results: The mean total Cost per Cow Calving observed in 133 North American herds was \$263 (std dev of \$81), with \$116 due to investment costs and \$147 due to disease consequence costs. Mean ketosis and mastitis costs/case across herds (\$102 and \$387, respectively) were very similar to recently published results. Significant factors associated with disease cost included average cow value, how waste milk is utilized, milk price, labor cost, market cow value, culling risk, and feed cost. The relative importance of each variable varied with the disease under consideration.

Conclusions: Considerable financial and management resources are devoted to mitigating the impacts of metabolic and infectious challenges that occur during The Vital 90 Days, but few have quantified the cost incurred by each cow. Indirect disease costs tended to be larger than direct disease costs and total consequence cost of disease was greater than the investment cost for the average dairy. Considerable variation exists across herds but use of the Economic Assessment Tool to generate the Vital 90™ Days cost per calving should help producers make better decisions and improve their understanding of how disease management impacts profitability.

Animal Health Economics

P04-004-020

Mastitis associated economic loss in Canadian dairy farms

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Objectives: Mastitis imposes significant economic losses on the Canadian dairy industry. Estimating the overall economic burden induced by this disease in a country is a crucial step to rationalize control measures to cover priorities. The main objective of the current study is to estimate the total cost of mastitis in Canada in 2014 and the reduction in losses that could be gained under various putative udder health improvement scenarios.

Materials and Methods: An economic calculation model was developed for computing the farm level costs using cow-level data. All remarkable components of economic losses due to mastitis were listed according to a review by Halasa et al. (2007). These components included milk losses, discarded milk, drugs, veterinary visits, diagnosis, product quality, labor, culling, death, material and investments. Costs related to practices used strictly for mastitis prevention (e.g. dry cow therapy, teat disinfection) were taken into account in this later component. For each component, equations required to calculate the losses due to that component were prepared. The model was designed so it can be adapted for systems with and without supply management (milk quota). To feed the model, Dairy Herd Improvement (DHI) and clinical mastitis (CM) data can be used as well as data on various dairy production inputs and outputs.

Results: This model will be applied to all dairy producers in the 10 Canadian provinces to compute costs at the provincial and national levels. In the current study, required data will be obtained using historical DHI data from the 9,600 Canadian dairies currently enrolled in milk recording programs. DHI data will be simulated for the 2,400 non-DHI dairies by drawing a number of herds with replacement within the DHI herds. The CM distribution from the National cohort of Dairy Farms of the Canadian Bovine Mastitis and Milk Quality Research Network will be used in the model (Reyher et al. 2011). A sensitivity analysis will be used to evaluate the impact of a potential reduction in CM incidence on the total cost, since no recent studies have been conducted on this topic in Canada. Some values will be obtained from a questionnaire which will be administered to 374 Canadian dairy producers participating in an ongoing study, the Canadian National Dairy Study 2015. In addition, estimated cost of production reported in an annual study conducted by the Canadian Dairy Commission will be used. Costs will be reported per year at the provincial and national levels as total mastitis costs, costs due to subclinical mastitis and CM independently, average mastitis costs per milking cow, average costs of subclinical mastitis per milking cow, average CM cost per milking cow, and average CM cost per affected cow.

Conclusions: This study will be the first to provide an estimate of mastitis costs in Canada and to report the attributed component costs. Furthermore, the developed economic model can be used to assess changes over time at the national level and can be adapted for use in different countries with or without a supply management system.

Acknowledgment: This project was supported by a contribution from the Dairy Research Cluster Initiative (Dairy Farmers of Canada, Agriculture and Agri-Food Canada, the Canadian Dairy Network and the Canadian Dairy Commission).

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Animal Health Economics

P04-004-021

Annual cost of sanitation of farms with bovine brucellosis in Uruguay

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Objectives: In Uruguay, the program of Bovine Brucellosis (BBr) is based on the principle of testing and slaughtering. Individual prevalence is estimated at 0.2%, reaching 0.8% at farm level. Currently, there are 344 farms under quarantine in the country. An "insurance system" compensates farmers for the price differential received from the slaughterhouse, for the sacrifice of positive animals.

The objective was estimating the annual cost of the sanitary measures applied to clean BBr hotbeds in Uruguay.

Materials and Methods: A stochastic simulation model was built, stratifying by farm type and herd size. The inputs were: number of new foci, number of precedent foci, herd size, per-head costs of bleeding and serologic diagnosis, cost of vaccine, and the amounts covered by the insurance for both dairy and beef cattle. Information System for Animal Health data were used to define the distributions of foci per stratum and initial prevalence at detection. The number of farms per stratum and herd population used to define the probability distributions was obtained from DICOSE. It was assumed serology every 120 days. The output of the model included total annual country-level costs of the sanitary program, including bleeding, diagnosis, and vaccination, and total compensation paid to farmers for the slaughter of positive animals in all existing foci.

Results: Serology costs averaged US\$ 725,000, corresponding to 137,460 serology, of 68730 cattle.

Vaccine application averaged US\$ 350,000, corresponding to 123338 cattle vaccinated.

Compensation for the slaughter of cattle reactors have averaged US \$ 1,290,000 that came from a number of cattle compensated 6953, 96% of which correspond to beef and dairy cattle 4%.

The estimated average annual total cost for serology, vaccination and compensation for slaughtered cattle reactors amounting US\$ 2,360,000. Ninety-five percent of the iterations performed by the model ranged from US\$ 1,558,000 to US\$ 3,504,000.

Conclusions: Considering the estimates of sanitation costs along with the annual costs of the insurance allowed the inclusion of new actions

to be covered by the latter, as the cost of foci sanitation, the serology tracing and vaccination of neighboring farms.

Comments: Once detected the infection in a herd, any movement of susceptible animals other than for slaughter is forbidden. All sites epidemiologically related to the interdicted herd are traced and investigated. All females are vaccinated. A periodical serology with elimination of reactors is performed until two subsequent negative rounds are obtained, with a minimum interval of four months.

Animal Health Economics

P04-004-022

A simple method to calculate the cost of mastitis under Spanish field conditions

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Objectives: The calculation of the cost of mastitis is complex and has been associated with multiple factors: the disease (prevalence, severity, effects, aetiology, concurrent disorders, etc.), the animal (lactation status, age, productive level), the setting (treatment protocols, methods of prevention, elimination, management), and prices (value of different expenses, price of milk, penalties and premiums). The complexity and heterogeneity of the many variables involved and their interactions promote duplication of effects and errors, and affect the credibility of results.

Materials and Methods: When trying to calculate the cost of mastitis at field level, simplicity and clarity are important, so we calculate the economic cost on the basis of 3 factors:

- 1.- Expense associated with treatment:
It takes into account the appearance of new cases of clinical mastitis (CM), the price of milk and the productive level of the farm, and the days that milk is discarded (it is assumed for 8 days, and none is used to feed calves).
- 2.- Losses in quality premium incentives
Part of the European dairy industry penalises or rewards the milk price on the basis of somatic cell counts (SCC). Therefore, the price of milk is strongly affected by the incidence of mastitis.
- 3.- Production losses due to high SCC.
There is a strong correlation between inflammatory processes like mastitis and the reduction in the functionality of the glandular parenchyma of the udder. The linear score (LS) is a method of interpreting SCC that is strongly associated to milk production

Results: In the following table this methodology is applied to a farm with 100 milking cows and 11,200 L/cow/year.

Table 1: Cost per clinical case

A	Average medicine costs (€)	20 €/case
B	Number of days for discarded milk (days)	8 days
C	Average production (L/cow/day)	35 L
D	Average milk price (€/L)	0.358 €.
E	Cost of professional fees (€)	20 €/case
F	Monthly percentage of new cases (%)	2 %
G	Number of cows per farm (No)	100

H	BxCxD Discarded milk, value(€)	100.24 €
I	A+E+H Total losses per case(€)	140.24 €
J	GxFxH Cost per farm/month(€)	280.48 €
K	Jx12 Cost per farm per year (€)	3,365.76 €
L	K/G Impact per cow (€/cow/year)	33.7 €

Table 2: quality premium incentives

A	Basis for SCC premium (x 100 cells/ml)	< 200
B	Bonus quality premium(€/MT)	6 €
C	Basis for SCC penalty (x 100 cells/ml)	> 300
D	Penalization (€/Tm)	-6 €
E	Monthly production supplied to industry (MT)	94
F	Number of animals	100
G	D-B Class A differential (€/MT)	-12 €
H	G*E Monthly loss (€/month)	-1,128 €/month
I	H x 12 Annual loss (€/year)	-13,536 €
J	I/F Impact per cow €/cow/year)	135 €

Table 3: Milk losses due to a high SCC

A	Total cows (No.)	100
B	Annual production (t)	1,120
C	Mean annual SCC (cells/ml)	320
D	LS	5
E	Milk price (€/L)	0.358
F	Average feed cost (€/L)	0.200
G	Annual estimated losses according to LS (L/cow/year)	540
H	G x A Total milk loss (L)	54,000
I	E-F Economic cost of milk not produced (€/L)	0.158
J	H x I Estimated annual loss (€/year)	-8,532 €
K	J/A Impact per cow (€/cow/year)	85.32 €

Conclusions: There is widespread agreement between specialists and researchers that for the majority of modern, advanced dairy farms, bovine mastitis is one of the major causes of economic losses due to disease and that therefore, quantification of these losses is an interesting and useful task. The exercise presented in this paper is a useful tool for the calculation of costs in field conditions in a simple and clear way.

Animal Health Economics

P04-004-023

Estimating the Impact of Mastitis and Metritis in Commercial Dairy Herds

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Objectives: The Vital 90™ Days span the 60 days prior to calving through 30 days in lactation. During this time, dairy cows experience negative energy balance and immune suppression as well as metabolic and infectious problems that often lead to clinical disease with increased

culling and mortality risks, diminished milk production and impaired reproduction. The objective of this project was to evaluate the impact of metritis and mastitis, occurring within the first 30 days of lactation, on production, reproduction and culling across the lactation and to evaluate the impact of disease misclassification on-farm on the resulting estimated disease impact.

Materials and Methods: On-farm dairy records covering a 12-month period of calving were collected from Elanco's Dairy Data Access System™, screened for consistency of recording and filtered to include only Holsteins. Animals were followed for 300 days after calving or until culled or calving again. Descriptive statistics were used to describe parity, twinning, age at first calving, average p305ME milk, and disease incidence. Multivariate nominal logistic regression was used to examine relationships between management or cow-level variables and risk of metritis and mastitis. Multivariate regression using the Fit Model platform and Standard Least Squares within JMP 12.1.0 software was used to evaluate the impact of metritis and mastitis on milk production while adjusting for covariates. Univariate survival plots for time to herd removal or time to pregnancy were plotted for each disease. Cox proportional hazards models were created to evaluate time to removal or time to pregnancy while accounting for important covariates. Risk ratios to compare the risk of culling or pregnancy were computed from the parameter estimates. Two individual herds that aggressively detect, record and treat postpartum disease were evaluated apart from the large data set as previously described to investigate the impact of disease misclassification. Within the smaller data set, cows recorded as having mild disease were relabeled as "normal" to mimic common behavior on commercial farms where only the most severe disease is recorded. The data were analyzed yet again to compare the estimated impacts.

Results: The final data set included more than 50,000 cow records. Cows that experienced mastitis within the first 30 days of lactation were predicted to lose ~2500 lbs of p305ME milk, had ~1.2 times higher odds of being culled by 300 days and had lower odds of becoming pregnant. Cows with metritis were predicted to lose 600 to 1000 lbs of p305ME milk and had similar odds of premature culling as with mastitis. However, cows with metritis had ~30% lower odds of conceiving by 300 d. Misclassifying cows that had mild disease into the normal category significantly underestimated the negative impact of metritis and mastitis on milk production, culling and reproductive performance.

Conclusions: Metritis and mastitis occurring in the first 30 days of lactation were associated with decreased milk production, increased culling and decreased reproductive performance. While the results found in this large scale observational study are similar to published research findings, biases introduced by failing to consistently and accurately identify and record disease such as metritis and mastitis are demonstrated to underestimate the true impact of postpartum disease.

Animal Health Economics

P04-004-024

Economic Analysis On Genetic Breeding Of Three Groups Dual Purpose Heifers Humid Subtropical Climate

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Objectives: This study was a collaborative research work between

the Autonomous University Benito Juarez of Oaxaca and the National Institute of Agricultural and Livestock Research whose goal was to estimate the cost of production of the three genetic groups of heifers dual purpose (meat and milk) from the birth to the first child to determine what is the most economical in terms of production costs.

Materials and Methods: The methodology used was that proposed by Aguilar et al. 2001. The economic data was analyzed from 2008 to 2010 at the Department of Livestock Production dual purpose (DP) "Las Margaritas" in Hueytamalco, Pue with an altitude of 500 msnm, it has a humid subtropical climate with average annual temperature 21°C and 90% average humidity. 202 cattle were handled on average each month in an area of 98 hectares. Also it was necessary to consult the records of income and expenditure of the resources generated and used in the production unit, also the books that record the inputs and outputs of all products warehouse and sales invoices and payments services. Variable costs (VC), fixed costs (CF) and total costs (CT) were determined from birth to birth, in this stage were included separately stages from birth to weaning and weaning to first birth.

Results: The results were \$ 1674.43 and \$ 715.57 for CV and CF respectively, covering the period from birth to weaning and was the same for all three genotypes. Weaning at first birth CVs were \$ 7,141.41 for genotype Holstein / Zebu (Hol / Ce), \$ 7554.68 for Brown Swiss x Cebu (SP / Ce) and \$ 6669.30 for the Simmental x Cebu (Sim / Ce) and CF \$ 485.69 for Hol / Ce, \$ 513.59 for SP / Ce and \$ 455.89 for Sim / Ce. From birth to the first delivery were \$ 10,019.10, \$ 10,460.21 and \$ 9,547.19 for Hol / Ce, SP / Ce and Sim / Ce, respectively. In economic terms, it was less expensive to produce replacement heifers Sim / Ce in the DP systems from weaning to the first delivery.

Conclusions: It can be concluded that the dual purpose was more economical to produce heifers at first calving genotype Sim / Ce, followed by Hol / Ce and finally the SP / Ce, which implies an obvious savings in production costs, however this does not suggest that the most profitable since the latter probably compensate with subsequent lactations investment used during development.

Animal Health Economics

P04-004-025

The Economic Impact Of Decreased Milk Production Due To Subclinical Mastitis In East German Dairy Herds

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Objectives: Nowadays mastitis has been identified as the most costly disease within the dairy industry. Economics of clinical mastitis have been subject to many recent studies. However, the subclinical form of mastitis has often been suspected to have a large influence on the revenue of a farm, but has been analysed rarely. According to very recent publications, 50,000 somatic cells/ml milk is used as a cut off value indicating inflammatory changes in the udder. The aim of this study was, to provide adequate information on the revenue loss of East-German dairy farms due to subclinical mastitis.

Materials and Methods: The data was taken from 12 farms in the Elbe-Elster region in the east of Germany. The total number of dairy cows surveyed in this study was 7,648 and their average daily milk production

was 31.98 kg. The individual cow's milk composition was tested by a Fossomatic analyser once a month, two to three times on the test day, according to the number of milking, in the period of June 2011 to June 2012. The average somatic cell count (SCC) of the surveyed farms' population was 284,320 cells/ml. The 50,000 cells/ml cut off value was used to calculate the decrease in milk production due to subclinical mastitis. In Germany and other milk exporting countries worldwide 250,000 somatic cells/ml is the recognised cut off point for supreme milk price payment. The average milk production of three cow groups (50,000-100,000, SCC/ml, 100,001-250,000 SCC/ml and over 250,000 SCC/ml) were compared to that of cows with an SCC less than 50,000 cells/ml.

Results: The results of this study show that subclinical mastitis causes a large milk yield depression. Even cows with an SCC higher than 50,000 cells/ml, but below 100,000 cells/ml showed a remarkable milk production loss of over 8%, resulting in 908.9 kg less milk during the lactation which really underlines that inflammatory changes must be present in cows showing a SCC of more than 50,000 cells/ml. Cows that had an average SCC between 100,001-250,000 cells/ml showed a reduced milk production of over 15%, which equals 1,650.05 kg milk loss during the lactation, even though this is still lower than the average SCC of 284,320 cells/ml. Average figures on an individual basis showed a daily decrease in milk production of 2.98 kg, 5.41 kg and 6.41 kg for a somatic cell count of 50.001-100,000 cells/ml, 100,001-250,000 cells/ml and over 250,001 cells/ml, respectively.

Based on the average milk price in the survey period, a single cow according to the aforementioned SCC classification showed a loss of € 294.85, € 536.80 and € 634.40 per lactation, respectively, in gross milk receipts. On an average herd level which was 637 dairy cows in this study, a total decrease of gross milk receipts was estimated to be almost € 241,000.00 per year. The feed costs were seen as fixed costs, hence, the possible savings owing to the lower milk production was not taken into account. The total loss due to both clinical and subclinical mastitis was amounted to more than € 273,000.00 a year in an average herd, taking the cost of premature disposal, discarded milk and reduced milk receipts into account.

Conclusions: The findings show that the reduced milk receipts due to lower milk production of cows with an SCC higher than 50,000 cells/ml, had the largest economic consequences, even larger than the generally accepted cut off value of 100,000 cells/ml for subclinical mastitis. The subclinical forms of udder diseases are often hard to distinguish for herd managers and accordingly fall into the responsibility of the veterinarian. They also often cause a far larger economic damage than the clinical forms, which is underlined by this study, hence they need far more attention.

Animal Health Economics

P04-004-026

Sustainability indicators of the family milk production

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Objectives: Economic, social and environmental factors were evaluated in eighteen family dairy systems in México highland, in order

to characterize their production systems and to know their level of sustainability.

Materials and Methods: "Frame for the evaluation of the management systems based on sustainable indicators (MESMIS in Spanish)" method was used to characterize production systems and to identify, in a practical way, the strengths and weaknesses of them.

Eighteen family dairy farms were evaluated in communities of the Mexican highlands, in the state of Michoacán; where the weather is tempered (14.1 to 29.9°C) and has a rain season in summer (897.7 mm annual rainfall). The study was performed from January to May.

The benchmarks selected in order to evaluate the farms were efficiency, quality, management practices, system continuity, the ability to change, middleman, outside supplies, incomes, job generation, self consumptions, quality of life and organization. As part of these indicators, the next variables were evaluated: milk yield, production cost of 1 L of milk, physicochemical characteristic of the soil, manure management, volume of the milk sales, marketing sales, adoption and implementation of technologies, gross marketing margin, consumption of outside supplies, type and quantity of incomes, use of wage labor, use of family workforce, self consumption of milk, public service access with type and structure and proper level of decision making capacity.

Results: The main values based on the criteria evaluated were the indicators of perception in the farmers, use of wage labor, soil characteristics and milk self consumptions, which finally leads to social stability in these communities.

It was observed that the application of some technologies allows strengthen the criteria of sustainability in the three evaluated areas (economical, social and environmental)

It was found that the production dairy systems in Maravatío, Michoacán, tend to the sustainability as long as the management of their components is performed in an integral way, with constant technological innovations, knowledge, social and practices materials.

Finally, three different marketing channels were identified in all of them, which allows them to have a gross marketing margin of 28.7%

Conclusions: In conclusion, under the methodology of MESMIS, all the factors involved in the small farms sustainable way of life can be evaluated with accuracy.

In this study, it was found that the small rural farm systems of this Mexican region has milk production as their main economic activity, which besides self consumption, allows them to have an important economic income for the survival way of life of this rural families with his own natural resources, with economical, social and environmental sustainability.

Animal Welfare

P02-002-001

Examination of the responsiveness of the leukocyte environment in beef calves to weaning stress using molecular biomarkers

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Objectives: The molecular mechanisms by which stress induces the development of pathologies remains unclear. In cattle, a number of necessary husbandry practices have been shown to activate the stress response, yet very little is known about the impact these have at the molecular level. The objectives of the study were i), to characterise, in male and female beef calves, the immune response to weaning stress in bovine leukocytes at the physiological and molecular levels, and ii), assess the difference between calves weaned in the presence of the dam and those weaned and penned away from the dam.

Materials and Methods: Twenty-eight clinically healthy, three-quarter bred Simmental beef calves were housed indoors on slatted floor pens with their dams on day (d) -28 of the study. The calves were offered a new diet of grass silage and had free access to concentrates. At weaning (d 0), the cows were removed and the calves were regrouped, mimicking social reorganisation, and allocated to one of four treatments: (i) females weaned beside the dam (n = 7; mean weight (s.d.) 267.3 (45.1) kg; mean age (s.d.) 227 (17.9) d), (ii) females weaned away from dam (n = 7; mean weight (s.d.) 274.1 (35.3) kg; mean age (s.d.) 224 (25.1) d), (iii) males weaned beside the dam (n = 7; mean weight (s.d.) 270.6 (69.8) kg; mean age (s.d.) 197 (38.1) d), or (iv) males weaned away from dam (n = 7; mean weight (s.d.) 267.9 (82.8) kg; mean age (s.d.) 209 (37.3) d). Treatments (i) and (iii) were assigned to pens adjacent to their dam. A metal grid fence prevented suckling whereas visual and auditory contact was allowed. Treatments (ii) and (iv) were assigned to pens sufficiently distant from their dam to prevent any visual or auditory contact. On d -3, 0, 1, 2, 3, 7, and 11 relative to weaning (d 0), blood samples were collected for haematology and gene expression profiling. Real-time qPCR was performed using the Applied Biosystems 7500 FAST RT-PCR equipment v2.0.1 using 1 µL of cDNA in a 20 µL reaction. Haematological and relative gene expression data were analysed (2x2 factorial design) using the PROC MIXED procedure of SAS (9.3).

Results: There was no effect (P>0.05) of gender, location and time or their interaction on total leukocyte or monocyte number. There was a location x time interaction (P<0.01) but no effect (P>0.05) of gender for total neutrophil number. Following weaning, neutrophil number increased in all calves (P<0.001) but the increase was greater (P<0.001) in calves weaned away from the dam than those weaned beside the dam. Neutrophil number returned to baseline by d 11. Lymphocyte number decreased (P<0.05) from baseline on d 2 and remained lower throughout the study. There was a gender x location interaction (P<0.01) for lymphocyte number, whereby away males had lower (P<0.05) lymphocyte numbers than near males and the opposite (P<0.01) occurred with females. There was no effect (P>0.05) of gender, location and time or their interaction on expression of IL-4 and lymphotoxin, and no effect (P>0.05) of location on IL-1β, IL-2, IL-8, IFN-γ and TNFα. Expression of IL-1β increased over 2-fold (P<0.001) from baseline on d 1 following weaning and remained increased on d 3 and 7. There was a gender x time interaction (P<0.05) for expression of IL-2, whereby an increase (P<0.05) from baseline occurred in females on d 1 but not in males. Female calves had greater (P<0.05) expression of IL-8 versus male calves. Expression of IL-8 increased more than 2-fold (P<0.001) from baseline on d 1 and remained elevated (P<0.05) on d 3. There was

no effect (P>0.05) of gender, location and time or their interaction on expression of NFκB2, p21 and haptoglobin.

Conclusions: Concerning management at weaning, it is concluded that suckled beef calves may benefit from a weaning strategy where they are allowed contact with the dam but prevented from suckling for a number of days before total separation occurs. The data also show an effect of gender in differential gene expression in response to stress with IL-2, IFN-γ and TNFα having increased expression in female calves compared with male calves. Furthermore, this study has established that a number of robust molecular biomarkers for weaning stress exist including: IL-1β, IFN-γ, TLR4, GRα and Fas.

Comments: This study utilised a model that was designed with the objective of isolating the maternal-offspring separation and social reorganisation occurring at weaning time, in order to characterise the weaning response without the associated stress resulting from simultaneous housing and novel handling. For this study, RT-qPCR analyses together with haematological profiles were examined to compare gene expression in blood leukocytes of weaned calves over a period of 7 days.

Animal Welfare

P02-002-002

The impact of insufficient winter feed on morbidity and mortality in Irish cattle and sheep in 2013

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Objectives: Anecdotal evidence from a range of sources indicated a substantial increase in animal mortality in Ireland in the winter/spring period spanning 2012/13. This was generally ascribed to the shortage of feed and the prolonged winter feeding period consequent to poor weather conditions in the summer of 2012 and the spring of 2013. The objective of this study was to evaluate available data in an attempt to objectively quantify the impact of the fodder shortage on mortality and morbidity in the Irish cattle and sheep population.

Materials and Methods: Bovine mortality data for the period January 1st to April 30th in both 2012 and 2013 was extracted from the Department of Agriculture, Food & the Marine (DAFM) Animal Identification and Movement (AIM) system, with cattle categorised by age group. The DAFM Laboratory Information Management System (LIMS) was used to compare the numbers of ovine and bovine carcasses submitted to Regional Veterinary Laboratories (RVLs) for post mortem during the study periods in both years to study mortality. Changes in morbidity were estimated using submissions of calf faecal samples to RVLs to screen for pathogens causing diarrhoea and serum samples submitted to evaluate colostral antibody status. An online survey of all Irish private veterinary practitioner clients of the RVLs gathered their opinions on the cause and impact of the event.

Results: There was an increase of 37,838 (31.4%) in the number of on-farm cattle deaths reported to the AIM database but this was not uniformly distributed across age categories. There was a 28% increase in the number of bovine carcasses submitted to RVLs, and a 35% increase in the number of ovine carcasses submitted. There were 8% fewer bovine foetuses but 47% more ovine foetuses submitted.

There was an increase of more than 15% in the number of submissions of faecal samples from calves affected by diarrhoea, with a marked increase

in the relative frequency of detection of rotavirus (from 40% to 66%) and a slight increase in the relative frequency of detection of Cryptosporidium (from 27% to 29%). The percentage of animals with a low ZST test reading (which is interpreted as a failure of passive transfer) increased from 24% to 53%. Deaths in sheep due to liver fluke (*Fasciola hepatica*) infestation increased.

Eighty seven (87) of the approximately 900 private veterinary practices (PVPs) that were invited responded to an online survey. An increase in the number of sick calves treated was reported by 58.6% of respondents. 47% of respondents said the response to treatment was worse in 2013 than in previous years, while 43.2%. Almost 80% (78.9%) of respondents reported that they had attended more recumbent cows in the spring of 2013. Reduced fertility was observed among autumn and spring calving herds. Liver fluke was observed as a particular issue for sheep. Almost 73% suggested veterinary knowledge transfer initiatives had a role in dealing with such problems in the future.

Conclusions: There was a significant increase in mortality among cattle in the first four months of 2013, compared to the corresponding period in 2012. The mortality in cattle seems to have been worst among animals sired by beef bulls. Poorer quality colostrum as a result of insufficient energy and crude protein in the diet of dry cows is likely to be a significant predisposing factor to increased disease and mortality among calves in the first month of life. Nutrition plays a pivotal role in the immune response and negative energy balance can contribute to immunosuppression. Such immunosuppression will predispose to increased disease and death.

Animal Welfare

P02-002-003

Italian on-farm welfare assessment protocol for dairy cows: results from the last 4-years activities

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Objectives: Over the last 10 years farm animal welfare has been of increasing interest for public opinion; consumers are very sensitive to how farm animals are reared and they are becoming more and more distrustful about farmers' practices. Challenge for scientific community and public institutions is to provide an objective tool for identifying if animal welfare is guaranteed or not. The Italian National Animal Welfare Reference Centre (CReNBA) based in the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER) in Northern Italy is trying to meet this challenge applying an on-farm welfare assessment protocol for dairy cows.

Materials and Methods: The CReNBA dairy cow welfare assessment protocol is based on scientific findings from Welfare Quality Project®, European Food Safety Authority (EFSA) publications on risk assessment related to dairy cow welfare and draft regulation concerning cattle (Strasbourg revised version No 8, 09/2009). The method takes into account also some minimum legal requirements (Council Directives 98/58/EC and 2008/119/EC). It consists of both management- and resource-based measures (Area A-Management and personnel, 23 indicators; Area B-Structures and equipments, 29 indicators) and animal-based measures (Area C, 18 indicators) such as: avoidance distance, body condition score, cleanliness of animals, lameness, integument alterations, udder health, mortality rate (cows and calves),

mutilations. Each assessment parameter has three choice options: unacceptable (hazard), acceptable and excellent (benefit). Parameters have different weighted scores given by an expert opinion consultation, based on their importance in decreasing or improving animal welfare. The overall welfare score results from adding partial scores of each Area and is expressed on a scale 0-100%. The protocol has been checked for inter-operators agreement. Application of this protocol by a skilled operator takes about 2 hours in a 100-lactating-cows-herd. From 2012 to 11/2015, 1047 Italian dairy herds in loose housing system have been assessed for animal welfare by 104 veterinarians that attended a specific training course at CReNBA. Farms were selected all around Italy for research activities and private consultation purposes.

Results: Dairy herds were characterized by an average number of 252.7 animals (min 7-max 2736), 116.3 lactating cows (min 4-max 1135) and 27.9 kg/per cow as daily milk production (min 10-max 41). The recorded overall welfare scores (WS) ranged from 36.18% to 95.90%, and most of them were very high (first quartile: 63.84%; median: 70.42%; third quartile: 77.31%). Herds with overall WS under the median value (70.42%) were found to be smaller and less efficient in milk production compared to herds that gained a WS >70.42%: statistically significant differences were found in average herd size (234 vs 271 animals, respectively; P-value = 0.0049), average number of lactating cows (108 vs 124.5 cows, respectively; P-value = 0.0053) and average daily milk production (26.8 vs 29.02 kg milk per cow/day, respectively; P-value <0.001). There were positive Pearson correlations between partial scores given to Area C (animal-based measures) and Area A (indicators about managerial factors; $\rho = 0.446$) and Area B (indicators about structural factors; $\rho = 0.382$). Despite very good results on animal welfare assessment, most of the farms (52.05%) had at least one non-compliance with minimum requirements provided by the law in force, mainly for calves under 6 months of age: the most frequent non-compliances were absence of contact between calves in single cages (349 farms) and disbudding performed after 21 days of age (226 farms).

Conclusions: The CReNBA dairy cow welfare assessment protocol is a reliable, timesaving, repeatable and scientifically-based tool to evaluate animal welfare level on-farm. Data collected in 1047 dairy herds showed positive information about dairy cow welfare in Italy. Herds with higher welfare levels were found to have more animals and higher production, as dairy cows in good welfare conditions usually have better performances. A stronger correlation was found between managerial factors (Area A) and welfare consequences on cows (Area C) meaning that skilful stockmanship can have larger effects in promoting animal welfare than housing structures (Area B).

Comments: For further information, please visit <http://goo.gl/iAtG4o>

Animal Welfare

P02-002-004

The effects of 6, 12, 30 or 48 hours of road transport on the physiology and behaviour of yearling beef cattle destined for a feedlot in Australia

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Objectives: In northern Australia, livestock can be transported by road over considerable distances and durations. Although industry experience suggested that healthy cattle adapted to local conditions tolerated such

transport well, the aim of this study was to quantify the impact of transport duration on behavioural and physiological indicators of cattle welfare, in yearling cattle transported under controlled conditions for 6, 12, 30 or 48 hours from farm to feedlot entry.

Materials and Methods: Composite *Bos taurus* x *Bos indicus* heifers (n=480; mean liveweight 383.5 ± 35.3 kg) were used for the experiment. The design comprised 4 transport duration treatments of 6, 12 and 30 and 48 h and these were replicated over 2 weeks. The cattle were transported from their backgrounding property to the feedlot using single trailer commercial trucks. The departure times were staggered to allow the trucks to arrive at the feedlot on the same day. On arrival, the cattle were allocated to pens with access to hay and water. Detailed measurements including liveweight, blood chemistry and haematology were collected on 15 focal animals on each vehicle (n=30 per treatment) pre-transport, immediately on arrival and 24 and 72 h after arrival. Rectal temperature was logged over the entire transport and 72 h post-transport phases. Behaviour was monitored over the immediate 6-7 h post-transport period for the 12- and 48-h transport treatments. After the 72-h recovery phase, the cattle were regrouped and finished in the feedlot over 43 days prior to slaughter.

Results: Blood variables were significantly altered by transport but generally remained within normal ranges. Blood urea nitrogen was elevated at 0 h compared with 24 and 72 h post-transport. Animals transported for 48 h in week 2 recorded the highest BUN after transport (22 mg/dL). Other hydration indicators- total protein and albumin were similar, with a decrease during the first 24 h post transport, and a duration × week × time interaction. Creatine kinase was increased following transport (694 U/L) and then declined over 72h, with no effects of transport duration. β-hydroxybutyrate was not increased by transport. Cortisol exhibited a significant transport duration × time effect. At unloading, cortisol was highest in animals transported for 48 h (93 nmol/L), followed by animals transported for 30 h (74 nmol/L), with animals from the 6- and 12-h treatments lower (~62 nmol/L) and not different from each other. In each case post-transport cortisol was lower than immediately prior to loading. Similarly, the combination of loading and initial stages of transport were the most stressful as indicated by a sharp rise in rectal temperature. There was positive trend between transport duration and liveweight loss however, the differences between the treatments were not always significant. The cattle recovered the majority (95 – 98%) of the weight lost through transport by 72 h after transport. Cattle transported 48 h spent significantly more time lying during the initial 3 h post-transport phase but not thereafter. Transport duration had no effect on feedlot average daily gain.

Conclusions: Although long-distance transport as practised by industry in northern Australia represents a physiological challenge for the animals, the data suggest that healthy cattle can respond and remain within acceptable physiological limits. The caveat is that these results apply to healthy, well-fed animals undergoing good practice handling and transport procedures. Cattle that are compromised by undernutrition, age or reproductive status, or by extreme climatic conditions, should only be transported if they are fit for the intended journey.

Animal Welfare

P02-002-005

Heart rate, heart rate variability and fecal glucocorticoid metabolites of dairy cows before and after changeover to an automatic milking system

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Objectives: A better comprehension of animal stress is a key aspect in welfare assessment. Continuous monitoring of the tone of the autonomic nervous system helps to identify both the biological and the environmental elements of a stress situation. In dairy cows, the type of milking – notably the human presence and handling it involves - is considered to be one of the main influential factors. The aim of the study was to measure the effects of a newly installed automatic milking system (AMS) on the welfare – as reflected in the changes in the autonomic nervous activity - of the cows at a Hungarian dairy farm.

Materials and Methods: The study was performed on a small scale (80 lactating cows) dairy farm where the conventional 2x5 herringbone milking parlour was changed to a new DeLaval VMS type AMS. In the first study period (March), when the conventional milking system was in operation, 27 clinically healthy Holstein Friesian dairy cows (age: 4.8±1.6 yrs, parity: 2.6±0.5, days in milk: 213.3±36.4) were examined. In the second part of the study (May) the same animals were studied, 2 months after the changeover to AMS. In this latter period, data from 18 of the 27 animals were analyzed.

Heart rate (HR) was recorded using a Polar RS800 CX recording system (Polar Oy Kempele, Finland). During the conventional milking period, the HR monitors were fitted on the animals after the morning milking and recordings lasted until returning from the evening milking (approx. between 8 am and 7 pm). During the AMS period, the electrodes were attached in the morning hours and detached after a 24-hour recording session. The Kubios HRV software (University of Kuopio, Finland) was used for heart rate variability (HRV) analysis. The SD2/SD1, HF, LF/HF measures of HRV were calculated in the periods of 1) lying and standing in the barn, 2) prior to the milking (driving and waiting) and 3) during the milking (preparation, milking, waiting for exit). Fecal samples were taken from the animals in the morning and in the evening on the day they were monitored, for laboratory determination of glucocorticoid concentration by RIA method.

Results: HR and HRV parameters measured when the animals were in their barns, lying or standing, showed no difference before and after the changeover. There were, however, significant differences in all parameters measured in the time period when the animals were driven by the stockperson and waiting for milking in the parlour, compared to the 'self-driven' and relatively calmer waiting period at the AMS. Area under the curves (AUC) of heart rate (mean ± SE: 48.9±12.0 vs. 25.9±7.6 min⁻¹, P=0.0217), SD1/SD2 (14.8±3.9 vs. 0.6±2.49, P=0.0009), LF/HF (12.6±0.4 vs. 9.2±0.1, P=0.033) were higher, while HF was lower (-23.1±13.6 vs. -5.3±7.3 normalized unit, P=0.0245) indicating higher sympathetic tone in the pre-milking period in the parlour milking routine compared to the AMS. During the milking and the post-milking waiting period (which was very short in AMS) the AUC values of SD2/SD1 (9.4±3.8 vs. -2.9±2.6, P=0.0325), LF/HF (20.7±1.9 vs. 13.7±1.4, P=0.026) were higher, while HF was lower (-29.2±13.4 vs. -0.8±4.9 n.u., P=0.0145) indicating a higher sympathetic tone in the conventional milking period compared to AMS. The HR did not differ during and after milking. The fecal corticosteroid concentrations were significantly higher both at the morning (54.5±3.1 vs. 20.6±1.8 ng/g, P=0.0038) and at the evening (58.2±4.3 vs. 19.0±1.9 ng/g, P=0.0034) sampling during the conventional milking routine.

Conclusions: Periods of frequent interaction with humans – and especially rough handling – are potential sources of stress for dairy cows,

as shown by a higher sympathetic tone. Freedom of moving and being milked voluntarily can significantly improve animal welfare on farms with automatic milking systems.

Animal Welfare

P02-002-006

Comparison of mortality rates in different categories of cattle during transport for slaughter

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Objectives: The death of animals during transport for slaughter is a major factor indicating the standard of welfare in transported animals. The aim of this study was to determine the mortality rate in different categories of cattle transported for slaughter in the Czech Republic in the period from 2009 to 2014.

Materials and Methods: Data summarizing the numbers collected from all cattle transport movements in the Czech Republic in the monitored period were gathered in the database of the Information Centre of the State Veterinary Administration. The percentages of transport deaths out of the total number of cattle transported within each category were calculated. On the basis of these results, the differences in transport-related mortality rates between individual categories of cattle in each year and for the entire monitored period were evaluated. All the data were analysed using the statistical package Unistat v. 6.5. Statistical comparisons between the frequencies of the categorical variables of interest were performed with the Chi-square test (with Yates correction when needed) within the contingency table procedure. Data concerning transport-related mortality rates in individual cattle categories were compared between the individual monitored years and also the differences in mortality rates among cattle categories were tested for each year. Data concerning mean mortality rates in individual cattle categories for the entire monitored period in the study were compared by a Kruskal-Wallis one-way ANOVA and subsequently by a Tukey-type test with ranked sums as a post hoc test for pairwise comparisons.

Results: In the monitored period, in total 1,552,574 head of cattle were transported for slaughter, out of which 1,935 (0.125%) died as a result of this transportation. During the monitored period, the overall mortality rate of cattle transported for slaughter in the Czech Republic declined from 0.333% in 2009 to 0.030% in 2014, which is a positive finding. However, highly significant ($p < 0.01$) differences were found among the transport-related mortality rates of individual categories of cattle. The highest mortality rate was found in calves (0.296%), followed by dairy cows (0.207%) and feeders (0.058%), and the lowest mortality was found in fattened cattle (0.017%). The mortality rate in calves transported for slaughter ranged from 1.045% to 0.063% in individual years with a significant decrease ($p = 0.044$) in the last year. The transport-related mortality in dairy cows ranged from 0.548% to 0.051% in individual years of the monitored period. The values fluctuated significantly ($p < 0.05$) among individual years. When comparing mortality rates among individual cattle categories, significantly ($p < 0.01$) higher levels of mortality were found in calves and dairy cows than in fattened cattle and feeders each year. Higher mortality rates in dairy cows and calves may be related to the fact that dairy cows and calves are excluded from breeding usually due to decreased performance, which may result from the animal's poor condition. Together with transport-related stress, this

may become a critical factor leading to impaired health and finally to the death of an animal.

Conclusions: It is obvious from the results that the age and category of animals transported play an important role in terms of animal welfare during the transportation process. The statistics of cattle deaths may further improve if the requirements for animal welfare are strictly observed by farmers, hauliers and slaughterhouse operators and the observance thereof controlled by the relevant administrative bodies with an emphasis on categories that are at higher risk of experiencing poor welfare during transport.

Animal Welfare

P02-002-007

Temporal relationship between milk production and meteorological variables in southern Brazil

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Objectives: The objective of this study was to evaluate the relationship between meteorological variables and milk production over a 15 year period in Holstein herds in Castro region, Paraná State, southern Brazil.

Materials and Methods: Milk collection data were available for a 15 year survey period and included animal identification, herd, milk control date, calving date, lactation order, days in milk, animal age, daily milk production and milk production per lactation and meteorological data included precipitation, environmental temperature, air relative humidity, wind speed, solar radiation, Piché evaporation index, temperature and humidity index (THI) and temperature and humidity index corrected for solar radiation and wind speed (corrected THI). The total data set was composed by 161,351 milk control data measured as milk/cow/day and 10,162 meteorological information, collected during 15 years period. Data were subjected to correlation analysis (CORR) and multivariate statistical tests including principal components (PRINCOMP) and step-by-step (STEPDISC) analyses, using the Statistical Analysis System (SAS®).

Results: Average daily milk production was 30.57 L/cow, where 74.74% of cows produced between 20 to 40 L/day with 30.19% of cows in second lactation aged between 37 to 60 months and between 101-200 days in milking. In addition, 67.18% of cows were milked twice a day and 32.82% milked three times a day. The highest average maximum temperature recorded during the 15 year period of observations did not exceed 30°C and the temperature and humidity indexes were maintained within acceptable values for animal thermal comfort (THI between 64 and 68). Correlations between milk production and meteorological variables during 15 year period and according to the climatic seasons were low. Although the minimum and maximum temperature variables showed higher relationships with milk production when compared with other variables, they were not responsible for a marked change in the milk production. When extremes were observed in temperature or humidity associated with solar radiation and wind speed, such as in Summer and in Winter, the corrected THI was important as an indicator of climatic welfare of lactating cows, even though this variable was not seen to influence milk production.

Conclusions: In Summer and Winter seasons, the temperature and humidity index corrected for solar radiation and wind speed showed great

importance as an indicator of climatic welfare of lactating cows, even though this variable was not seen to influence milk production. The Castro region, Parana, Brazil has adequate weather for milk production from high performance European dairy breeds.

Animal Welfare

P02-002-008

Quantification of extended characteristics of locomotor behavior in dairy cows

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Objectives: The objective of this study was to develop and validate a novel algorithm to monitor locomotor behavior of loose-housed dairy cows based on the output of the RumiWatch® pedometer. It was hypothesized that a novel algorithm of the RumiWatch® pedometer device can be developed that provides a high correlation of parameters of behaviour of dairy cows in both upright and lying positions between the output data of the pedometers and the data derived from temporarily staggered video analysis.

Materials and Methods: Data of locomotion were acquired by simultaneous pedometer measurements at a sampling rate of 10 Hz and video-recordings for manual observation later. The study consisted of 3 independent experiments. Experiment I was carried out to develop and validate the algorithm for lying behavior, experiment II for walking and standing behavior and experiment III for stride duration and stride length. The final version was validated, using the raw data, collected from cows not included in the development of the algorithm. Spearman correlation coefficients (r_s) were calculated between accelerometer variables and respective data derived from the video recordings (gold standard). Dichotomous data were expressed as the proportion of correctly detected events, and the overall difference for continuous data was expressed as the relative measurement error (RME).

Results: In all experiments, the mean difference between accelerometer data and respective gold standard was between 0% and 17%, and the correlation between respective data ranged from $r_s = 1$ to $r_s = 0.75$. The proportions for correctly detected events or bouts were 1 for stand ups, lie downs, standing bouts, and lying bouts and 0.99 for walking bouts. The relative measurement error and Spearman correlation coefficient for lying time were 0.09% and 1; for standing time, 4.7% and 0.96; for walking time, 17.12% and 0.96; for number of strides, 6.23% and 0.98; for stride duration, 6.65% and 0.75; and for stride length, 11.92% and 0.81, respectively.

Conclusions: The strong to very high correlations of the variables between visual observation and converted pedometer data indicate that the novel RumiWatch® algorithm may markedly improve automated livestock management systems for efficient health monitoring of dairy cows.

Animal Welfare

P02-002-009

Effect of an intravenous injection of flunixin on stress related parameters in healthy dairy cows

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Objectives: Previous studies investigated the effect of non-steroidal anti-inflammatory drugs (NSAID) on stress and pain in cows with various disease by evaluating behavioral changes and measuring cortisol. The efficacy of NSAID to lower cortisol and reduce behavioral changes has been proven. However, it remains unclear how much the injection effected the stress parameters and if NSAID influenced cortisol only by reducing pain or by effecting the cortisol metabolism itself. Therefore, the aim of this study was to investigate the effect of a single injection of NSAID on fecal cortisol concentration and standing and lying behavior in healthy dairy cows.

Materials and Methods: Twenty-four healthy (i.e., without metabolic or infectious disease or lameness) Holstein-Friesian dairy cows were enrolled 14 – 21d after dry-off in a double-blind, placebo-controlled, crossover trial. Upon enrolment all cows were fitted with a HOBO Pendant G Data Logger (Onset Computer Corporation, Pocasset, MA) to continuously record standing and lying behavior in 1-min intervals. Daily time lying and number of lying bouts was calculated starting with the day after enrolment. Four days after enrolment (t0) cows were fixed in tie stalls and received an intravenous injection in the jugular vein of either NSAID (i.e., 2.2 mg/kg flunixin, 2ml/45kg Parafunixin RPS, Bayer Animal Health, Monheim, Germany) or placebo (PLA; i.e., 2 ml/45kg normal saline) based on a randomization list. Fecal samples were collected to measure fecal cortisol metabolites (i.e., 11,17 dioxoandrostanes; DOA). DOA measurement is a well-established and feedback free method (i.e., minimal stress due to restraining and handling) to quantify stress in cows. Fecal samples were obtained from the rectum before treatment (t0) as well as 24, 36, 48, 60, 72 and 96h after treatment and frozen at -26°C till analysis. DOA was measured utilizing an 11-oxo-etiocholanolone enzyme immunoassay. 1 wk after first treatment cows received an injection of the substance that was not used in the 1st study week. Fecal samples were again collected on t0, t24, t36, t48, t60, t72 and t96.

Data were analyzed utilizing a generalized linear mixed model. Time after treatment was included as repeated factor and cows as random effect.

Results: Baseline DOA on t0 did not differ between 1st and 2nd study week (93.2 ± 38.4 ng/g; $P = 0.256$). While there was no difference between NSAID and PLA treated cows ($P = 0.284$), DOA concentration in all cows increased between 24 and 36h after injection (115.5 ± 51.8 ng/g; $P = 0.009$) and remained on an elevated level until 72h (111.7 ± 59.1 ng/g) after injection ($P > 0.05$). DOA baseline levels of t0 and t24 DOA were reattained 96h after injection ($P > 0.05$). Time lag between injection and an increased DOA level was consistent with previous studies that described a minimum of 8 to 16 h between an increase in blood cortisol coinciding with the stressor and an elevated concentration of fecal DOA. There was no effect of week (i.e., 1st or 2nd study week; $P = 0.997$) or parity ($P = 0.692$) on DOA concentration.

Before treatment average daily time lying was 750 ± 170 min with an average of 8.7 ± 3.6 lying bouts. On the day of injection average daily time lying decreased to 669 ± 165 min ($P = 0.003$) with an average number of lying bouts of 8.0 ± 3.2 ($P = 0.039$). Total time lying and the number of lying bouts reattained baseline values 1 day after injection and remained on these level throughout the study. While there was no effect of treatment (i.e., NSAID or PLA; $P = 0.241$) and study week ($P =$

0.707) on daily time lying, an interaction between parity and time relative to treatment was found ($P = 0.06$). Cows in 3rd or higher lactation lay 98 min less on the day of injection compared to the days before and after ($P < 0.001$). There was no effect in 1st or 2nd lactation cows ($P > 0.05$).

Conclusions: NSAID have no effect on cortisol metabolism in health dairy cows and do not influence standing and lying behavior. While a single intravenous injection and the associated fixation of the cows effect standing and lying behavior on the day of treatment, fecal glucocorticoid metabolites remain elevated up to 72h after injection. Further studies are necessary to investigate, why standing and lying behavior on the day of injection was only affected in older cows.

Animal Welfare

P02-002-010

Prevalence of production diseases in European organic dairy herds

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Objectives: Despite decades of research and efforts in cattle herd health, production diseases remain a serious problem in modern dairy operations. They compromise the welfare of cattle and result in severe economic losses. Organic farming aims at good animal welfare and seeks to realise that aim by setting minimum standards for housing, feeding and management. The purpose of this study was to determine the prevalence of production diseases in organic dairy herds in four European countries.

Materials and Methods: Data from organic dairy farms that took part in the IMPRO project (impro-dairy.eu) were analysed. All farms were visited once in 2013 for the assessment of general characteristics, resources, and management procedures. A specifically designed on-farm protocol was administered during the visits which included a face-to-face interview with the person in charge of herd health management. A representative sample of the lactating cows was scored for lameness according to the Welfare Quality[®] protocol for dairy cattle. Data from the national milk recording systems and animal movement databases were retrieved according to the specifics in each country. All records were transformed into a common data structure and analysed using a common R script. Herd prevalences were calculated using sets of herd-level indicators related to udder health, metabolism, reproduction and lameness. Subclinical mastitis (SM) was diagnosed if composite milk samples were above a somatic cell count of 100 000 cells/ml. Risk of ketosis (KET) was defined as a fat-to-protein ratio > 1.5 during the first 100 days of lactation. Risk of subacute ruminal acidosis (SARA) was defined as milk fat percentage < 3.0 after 30 days in milk. In terms of reproductive performance, the prevalence of prolonged calving intervals (PCI) > 400 days was calculated. Overall lameness (OL) prevalence was obtained by adding up moderate and severe lameness prevalence.

Results: The study enrolled a total of 192 farms, with 60, 23, 54 and 55 in Germany (DE), Spain (ES), France (FR) and Sweden (SE), respectively. Within the whole group the time since conversion to organic farming ranged from under 1 to 29 years with a median (m_{ALL}) of 7. Herds were largest in SE (m_{SE} 68.1 cow-years) and smallest in ES (m_{ES} 29.7) ranging from 7.4 to 376.5 cow-years. In all countries all lactating cows

had access to pasture except for DE where 23% of farms only provided an outdoor run. In 69.8% of all farms cows were kept in loose housing with cubicles. Automatic milking systems were used by 10% of farms in DE and 49.1% in SE, and not at all in ES and FR. On animal-level the predominant breeds across countries were Holstein (48.2%), Red Dairy Cattle (15.6%), and Simmental (9.9%). Milk yield varied between countries. The median energy-corrected yields were 6588, 5742, 6378, and 8979 kg in DE, ES, FR and SE respectively (range_{ALL} 2324 – 10 880 kg). Herd prevalence of SM ranged from 18.9 to 94.2% and was lowest in SE (m_{SE} 44.1%) and highest in ES (m_{ES} 57.5%). KET prevalence was lowest in ES (m_{ES} 6.2%) and highest in DE (m_{DE} 11.4%) ranging from 0 to 45%. Prevalence of SARA was lowest in SE (m_{SE} 2.2%) and highest in ES (m_{ES} 14.8%) with a range of 0 – 42.3%. PCI ranged from 4.4 to 90.5% and was highest in ES (m_{ES} 62.5%) and lowest in DE (m_{DE} 36.3%). Median prevalence of OL was 20.4%, 10%, 23.2%, and 4.17% in DE, ES, FR, and SE, respectively (range_{ALL} 0 – 79.3%).

Conclusions: In terms of production diseases there is a large variation amongst organic dairy farms in Europe with a considerable proportion of farms not meeting the aim of good animal health and welfare. This leads to the conclusion that process-related minimum standards for organic animal husbandry cannot be regarded as effective in that respect. One major achievement of this study was the description of the status quo on the basis of extensive data and harmonised methods. The results call for new, improved strategies to achieve outstanding animal health and welfare in organic dairy systems.

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Animal Welfare

P02-002-011

Comparison of efficacy and onset of intravenous regional and nerve block anesthesia in the distal hind limb of dairy cows

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Objectives: Claw lesions leading to lameness are frequent health disorders in dairy cows and require commonly surgical correction for treatment. As part of a pain management protocol, local anesthesia is necessary for such surgical interventions. Although, intravenous regional (IVRA) and nerve block anesthesia (NBA) are described in textbooks the efficacy and onset of these two techniques are not yet evaluated in dairy cows. Therefore, efficacy and onset of anesthesia in the distal hind limb after IVRA and NBA were tested by means of pain stimulation techniques.

Materials and Methods: Six healthy, non-lactating, non-pregnant German HF cows were used in a cross-over design study. In all cows IVRA and NBA were tested in lateral recumbency on a surgical tipping table. For

IVRA, 20 ml procaine (Procasel 2%, WDT, Germany) were injected into the V. digitalis dorsalis communis III or V. digitalis lateralis communis IV after application of an esmarch bandage in the metatarsal region. NBA was performed at four sites using about 15 ml procaine at each site (A: Superficial Fibular Nerve: directly beneath the skin subcutaneously at the dorsal hind leg proximally to the hock joint, B: Deep Fibular Nerve: at the dorso-medial surface of metatarsus inside the proximal region of the groove, C: Medial Plantar Metatarsal Nerve: at the middle of medial metatarsus preneurally above the superficial flexor tendon and D: Lateral Plantar Metatarsal Nerve: at the middle of lateral metatarsus preneurally above the superficial and deep flexor tendons). Anaesthesia was tested by assessment of thresholds of I) Electrical stimulation (Grass S48 Square Pulse Stimulator) at the dorso-lateral coronary band as well as soft skin of heel, and II) Mechanical pressure stimulation at the lateral and medial sites of the bulb of heel, flexor tendon and fetlock joint. Stimulation was performed before (control) and 5, 7.5, 10, 12.5, 15 and 20 min after anesthesia. Reaching of the threshold level was assumed when first, slight responses on stimulation such as claw movements were recognized. One cow was excluded from statistical evaluation, because 20 min after anesthesia electrical stimulation revealed no complete onset of anesthesia. Results were statistically evaluated by ANOVA for repeated measurements (SAS package Vers. 9.3).

Results: Before anesthesia reaction thresholds for electrical stimulation were in average 37 V (+/- 9, range 25 - 60) and for pressure stimulation 17 N (+/- 4, range 9 - 20). Twenty min after anesthesia in statistically evaluated cows, tested thresholds reached the preset maximum level of 150 V and 20 N for electrical and pressure stimulation, respectively.

However, the excluded cow showed after IVRA no reactions on pressure stimulation at 20 N but reacted on electrical stimulation at 120 V. After NBA all evaluated cows reached the maximum preset levels of electrical pain stimulation after 12.5 min at the heel and after 10 min at the coronary band. Maximum stimulation levels were reached not before 15 min after IVRA which was significantly delayed compared to NBA cows (group and group * time interaction effects $p < 0.05$).

Conclusions: IVRA and NBA lead generally to anesthesia in the distal hind limb of dairy cows. Because in one cow IVRA failed to induce full anesthesia, efficacy of both techniques need to be tested in a larger number of cows. Onset of anesthesia in the distal hind limb of cows is significantly delayed after IVRA compared to NBA. Surgical treatments of claw lesions in the field are commonly performed under time constraints. Therefore, the delay in the onset of anesthesia after IVRA may have practical relevance in the view of animal welfare, when surgical interventions are executed before full onset of anesthesia is achieved.

Keywords: Cattle, Claw, IVRA, NBA, Pain

Animal Welfare

P02-002-012

Monitoring welfare in practice on Dutch dairy farms

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Objectives: The Welfare Quality welfare assessment protocol[®] (WQ) is quite intensive and it takes a long time (up to a day) to assess a farm. Several other protocols have been designed and published that do not require a full day for a single farm. These have been compared with WQ. This in order to come to a less time consuming protocol that has a similar

outcome. This is important because an increase in welfare is correlated with a higher milk yield (Van Eerdenburg et al., 2013).

Materials and Methods: In this project 3 other welfare measuring protocols: Welzijnswijzer (=Welfare Indicator), Koekompas (=Cow Compass) and the Continue welzijns monitor (=Continuous Welfare Monitor), have been compared with WQ on 60 dairy farms in the Netherlands. Four veterinary practices made a list of their dairy farmer clients. Each was given a score from good to bad, based on the availability of good quality food & water, quality of housing, health and behaviour. Out of the lists, randomly, 60 farms were selected in such a way that in each practice there were 5 good-, 5 average- and 5 bad farms. The farms were visited within 2 weeks for all protocols in order to avoid changes in welfare status over time. The result of the WQ protocol was considered to be the reference and the other three were correlated with WQ (Pearson correlation in SPSS version 20.0). Not only at the level of the end score, but also at principle, criteria and indicator level.

Results: The results for WQ were: 3 farms with score Not Classified, 52 with score Acceptable and 5 Enhanced, no farm was scored Excellent. This implies that WQ does not have a proper discriminative capacity. Because of this, the correlations with the other protocols were very low and not statistically significant. In relation to the WQ endscore the Pearson correlation was calculated for the principle of Feeding 0.46; Housing 0.15; Health -0.07 and Behavior 0.47. Therefore, the original WQ was adapted in 3 ways: 1) Drinkers: If one of the drinkers is dirty, all drinkers are considered to be dirty. In the adapted protocol, therefore, a weighted score for cleanliness was introduced. A clean drinker scored 1, partially dirty 2 and dirty 3 points. The total for drinkers is divided by the average score for the cleanliness. 2) Integument alterations: The average number of HP, lesions and swellings per cow is used in the calculations. 3) Qualitative Behaviour Assessment (QBA): The QBA is seriously disputed (Bokkers et al., 2012) and, in the experience of the present study, very difficult to explain to the farmers. With the use of the QBA, farmers are not convinced that the result is something to be taken seriously. Therefore, the QBA was omitted.

A new score was calculated for the 60 farms: 22 farms scored Not Classified, 31 scored Acceptable and 7 Enhanced, no farms scored Excellent. The Pearson correlations of the 4 principles were: Feeding: 0.85; Housing: 0.45; Health: 0.99 and Behaviour: 0.99.

Conclusions: Assessing welfare of a group of animals can be done with animal based measures and environmental measures. Animal based measures, like behavior, reflect better what the status of the cow is, but need a substantial amount of time to assess, whereas measuring the environment can be done relatively fast. For some parameters there was a high correlation between the animal based and environmental measures.

It is proposed to change the WQ protocol according to the 3 adaptations described above. The newly developed protocol can be used as a screening tool for welfare problems and to improve the management on a dairy farm.

Animal Welfare

P02-002-013

Pre – Slaughter Assessment of the Welfare of Livestock at Three Abattoirs in Northern Nigeria

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Objectives: In Nigeria, most cattle are reared under extensive system in rural areas and spend long time in transportation between farms, market and slaughter places. Lack of good roads and poor transport system, have increased the difficulties of transportation of livestock in the country. Due to lack of trained personnel, animals are subjected to varying degrees of cruelty which have severe consequences on animal welfare, leading to reduction in meat quality. This study was conducted in order to evaluate the potential effects of pre-slaughter operations on welfare of animals at three municipal abattoirs in northern Nigeria.

Materials and Methods: Three municipal abattoirs in Bauchi, Kaduna and Nasarawa States in northern Nigeria were visited twice weekly for 12 months (May, 2013 – June, 2014). The operations considered include supply chain of animals, distances traveled, transit time, body condition score, and effect of trekking on physiological parameters of the animals slaughtered. Prevalence of bruising using gross assessment was determined in cattle, sheep and goats slaughtered. A pretested questionnaire was administered randomly to 150 animal health officers, livestock transporters and other personnel responsible for handling and inspection of animals at the three abattoirs. Blood samples were collected for haematological analysis from 50 each of cattle, sheep and goats during the slaughter period. Physiological parameters of cattle that trekked for 37 km to Lafia abattoir were measured.

Results: The result indicated that animals slaughtered at the three abattoirs were supplied from 21 different sources. Over 83% of all the animals slaughtered were goats in Bauchi and Lafia and 41% in Kaduna municipal abattoirs. Many of these animals have traveled distances of about 500km and spent up to 7 hours on transit. It was noticed that there were no appropriate loading facilities and animals are forced down from vehicles. It is worthy to note that the vehicles used were not designed for animal transport. Presence of abattoir equipment was only available at the Kaduna abattoir. Assessment of BCS showed that 4.2% of all the cattle slaughtered were emaciated, 26.1% moderate and 38.7% obese. Similar trend was observed in sheep and goats. There was poor knowledge of animal welfare among most of the personnel interviewed. The average prevalence of bruising in cattle, sheep and goats were 13.03 ± 0.20 , 10.66 ± 3.72 and 10.83 ± 1.76 respectively. Greater percentages of bruising were recorded on animals that traveled up to 100 km and above. There was no significant difference in PCV and Hb values between male and female cattle, sheep and goats slaughtered at Lafia and Bauchi abattoirs. However, there was a significant ($P < 0.001$) variation in blood parameters of male and female cattle and sheep slaughtered in Kaduna abattoir. There were also significant ($P < 0.001$) differences in body temperature and pulse rate in both male and female cattle before and after 37 km trekking.

Conclusions: Poor transport system; lack of abattoir infrastructure and lack of trained workers were identified as existing problems compromising animal welfare in the study area. It is therefore recommended that further research work concerning both transport and handling of livestock be conducted in a larger scale in Nigeria. This will be helpful in identifying solutions to the torment of animals during transport and at the abattoirs and the general improvement of animal welfare in Nigeria.

Animal Welfare

P02-002-014

Impact of meloxicam administration in cows prior to caesarean on the efficacy of transfer of passive immunity in calves

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Objectives: Some authors suggest that in case of clinical signs of reduced vitality, it is plausible that providing NSAID to calves could improve the time to standing and increase colostrum intake and therefore decrease the risk of failure of passive immune transfer. The objective of the current study was to assess the benefit of meloxicam prior to caesarean to cows on the efficacy of transfer of passive immunity under the assumption that providing analgesia on the dam could lead to an earlier or longer colostrum intake by its own calf.

Materials and Methods: The study was performed in Burgundy in France in 2 veterinary practices. 48 Charolaise's cows were included in the study. Each cow was randomly allocated and was either treated with meloxicam subcutaneously (0,5 mg/kg) ($n=22$) or received no analgesia ($n=26$) prior to caesarean. Classical left flank approach with local anaesthesia was used for each cow. For each cow, colostrum quality (based on Brix refractometer), delay between the end of the caesarean and the first spontaneous colostrum suckling (based on farmer observation) and the IgG content in the sera (based on Radial Immuno Diffusion) of their calf 24 hours after birth of cows were analysed. In a first step, univariate analysis was done to compare the similarity of the cows within the 2 groups (colostrum quality, delay before first suckling). In a second step, the level of IgG was compared between treatment groups using chi-square test. In a last step, a logistic regression was performed to explain the level of IgG (below or above 15 g/L) according to the treatment group, the parity of the dam, the quality of the colostrum and the delay before first suckling.

Results: No significant differences were observed in the quality of the colostrum nor in the delay between the end of the surgery and the first spontaneous suckling of the calf between treatment groups. However, the number of calves showing a better transfer of passive immunity (IgG content >15 g/L) was significantly higher among those originating from dams receiving meloxicam prior to caesarean. The results of the final logistic regression models indicated that the probability for a calf for having an efficient passive immunity transfer (>15 g/L) was significantly higher for calves born from a dam receiving meloxicam ($p=0.03$; OR=3.88 [1.01-14.96]) and for calves who suckled their dam within the first hour after birth vs > 4 hours ($p=0.02$; OR=5.90 [1.12-31.15]).

Conclusions: This original study confirms that pre-emptive analgesia in cows prior to caesarean leads also to a benefit for its own calf through an improved colostrum intake ensuring a more efficient transfer of passive immunity crucial for its short and long term survival. Additionally, this study confirms the crucial role of an early colostrum intake.

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Animal Welfare

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Interim results from a large-scale lameness prevalence study in Irish dairy herds.

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Objectives: The majority of Irish dairy cows are maintained in a pasture-based system. Paddocks are grazed in rotation and cows walk to and from milking twice daily. Abolition of EU milk quotas has led to increased herd sizes, enlargement of grazing platforms and an increase in the distances cows walk for milking. Existing research indicates that longer walking distances predisposes dairy cows to hoof disorders. The objectives of this study were to (i) document the prevalence of mobility abnormalities and foot lesions in a large population of dairy cows, and (ii) investigate associations between mobility scoring and the presence of a foot lesion.

Materials and Methods: In November 2014, 69 spring-calving pasture-based dairy farms in the Munster area of Ireland were recruited to the HealthyGenes project. The total number of cows available for study was 11,380 of varying breed (Holstein-Friesian, Jersey, Norwegian Red). All cows were mobility scored (MS) using the DairyCo four point scale (<http://www.dardni.gov.uk/dairycochart.pdf>) on two occasions (spring and autumn) by two assessors scoring to a consensus. Mobility score was dichotomised for statistical analysis; cows recording a MS of 0 or 1 were categorised as 'none or minor mobility impairment' and those recording a MS of 2 or 3 were categorised as 'moderate to severe mobility impairment'. Hoof-trimming was also completed on over 6,000 cows and each individual categorised on the basis of how many lesions were simultaneously present (e.g. 0, 1, 2, 3 etc.). Lesions were identified using the ICAR Claw Health Atlas (http://www.icar.org/Documents/ICAR_Claw_Health_Atlas.pdf).

Descriptive data were coded and analysed in Excel (MS Office 2010). All statistical analyses were completed in Stata version 12 (StataCorp, USA). Univariable logistic regression was used to examine associations between MS in spring and MS in autumn, MS and the presence of at least one hoof disorder, and MS and the number of foot lesions present. Foot lesions were characterised as mechanical (e.g. white line disease, bruising, ulceration) or infectious (e.g. digital dermatitis, infectious pododermatitis). The severity of each lesion was also recorded but was outside the scope of the current analysis.

Results: Interim data from 46 herds were available for this analysis. In spring, 4% of all cows recorded MSs of two or greater. The prevalence of moderately to severely lame cows increased significantly to 6.7% in autumn ($P=0.0004$). Regression analysis highlighted that cows in autumn were almost 10 times more likely ($OR=9.83$; $CI=7.43, 13.02$; $P<0.0001$) to record a MS of two or greater compared to spring. In both spring and autumn, cows with multiple lesions were more likely to record a MS of two or greater. Cows recording five simultaneous lesions were over four times more likely to have a higher MS than those with no foot lesion ($P<0.0001$). Cows with a MS ≥ 2 were over three times more likely to have at least one foot lesion than cows with a lesser MS ($P<0.0001$). Mechanical lesions ($n=9413$) were more highly prevalent than infectious lesions ($n=156$). Bruising, white line disease and overgrown digits were the most common lesions recorded.

Conclusions: The prevalence of moderate to severe lameness in the Irish dairy system compares favourably with more intensive, indoor systems internationally. The increased prevalence of cow lameness in autumn, however, is a cause for concern, and most likely reflects the cumulative effect of daily road walking as the lactation progresses. The

greater number of mechanical rather than infectious lesions recorded in this study lends support to this theory. Although infectious causes of lameness require on-going prevention, we suggest that roadway/yard design and maintenance should form the basis for lameness control programmes on Irish dairy farms.

Animal Welfare

P02-002-016

Haematology, Serum Glucose And Some Biomarkers Of Inflammation Of Some Domestic Ruminants' Lameness In Ibadan, Southwestern Nigeria

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Objectives: In Nigeria, lameness is not yet identified as much welfare problem and as such there is scarce information on its effects on haematology, levels of serum glucose and measures of inflammatory reaction in domestic ruminants. This study was therefore aimed at investigating the effects of lameness on the haematology, serum glucose levels and some biomarkers of inflammation in some lame cattle, sheep and goats in Ibadan, South western Nigeria. The study was carried out in various settlements such as cattle and small ruminants markets, few households and Veterinary Teaching Hospital University of Ibadan Nigeria.

Materials and Methods: The domestic ruminants randomly sampled included 1500 cattle, 300 sheep, and 2200 goats of various breeds having estimated ages ranging between 8 months to 6 years, using the method of dentition. The study was carried out over a period of one year (2013-2014). Whole blood and serum samples were collected when the clinical signs of lameness were obvious and assessed by visual locomotion scoring of ≥ 3 and all causes of the condition diagnosed in each ruminant. The haematological parameters (Packed Cell Volume, Red Blood Cell Count, Haemoglobin Concentration, Mean Corpuscular Volume and Mean Corpuscular Haemoglobin Concentration) were determined using standard laboratory techniques with calculations. C-reactive protein (CRP), Tumour Necrosis Factor (TNF- α) and levels of Interleukins 1 and 6 (IL 1 and 6) were determined using Water's 616/626 High Performance Liquid Chromatography system while the serum glucose and Serum Amyloid A (SAA) levels were determined using Technicon's rapid colorimetric wave auto-analyzer II system. All data obtained was analyzed using descriptive statistics and SPSS statistical package version 9.0 at a significance level of 5%.

Results: Infectious and traumatic conditions appeared to be the most common causes of lameness in the three species of domestic ruminants; males tended to be more predisposed to lameness than their female counterparts in all the three ruminant species. Adult cattle and goats tended to be more predisposed to lameness but lambs seemed to be more predisposed among sheep. Most erythrocyte parameters which were significantly ($P<0.05$) decreased in lame cattle and goats were not significantly ($P>0.05$) different in lame sheep compared to normal values. There were significant ($P<0.05$) changes in the total and differential leucocyte parameters in the three species of lame domestic ruminants. All the selected inflammatory biomarkers (CRP, SAA, TNF- α , IL1 and IL6) were significantly ($P<0.05$) increased while the serum glucose level was significantly ($P<0.05$) reduced in the lame cattle, sheep and goats compared to the apparently healthy animals.

Conclusions: Therefore, Nigerian ruminant farmers and owners are now aware that lameness is a problem among all domestic ruminants with mechanical and infectious causes having deleterious effects on

adult large ruminants and goats. The increased inflammatory reactions and hypoglycemia in affected domestic ruminants are highlighted.

Comments: Key words: Haematology; Serum Glucose, Biomarkers of Inflammation; Domestic ruminants

Animal Welfare

P02-002-017

Physiological and hematological parameters of cattle

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Objectives: Reference values used in tropical and subtropical regions are based on cattle raised in temperate climate and those in Brazil are subject to different environmental stressors. Therefore, the aim of the present work was to define reference values for blood and physiological parameters for cattle in Brazil.

Materials and Methods: A database with 355 observations of cattle in the South, Midwest and Northeast region of Brazil with information on time of day, breed, physiological and hematological characteristics and environmental variables. The Temperature and Humidity Index (THI) was calculated based on the climatic data at the time of each experiment using the following equation according to Thom (1959): $THI = \frac{1}{16} TDB + \frac{5}{8} TWB - 4$; Where: TDB = Dry Bulb Temperature (°C); TWB = Wet Bulb Temperature (°C);

The environmental condition was classified according to the level of stress and was considered stressful THI above 70 and not stressful THI at or below 70 (Hahn et al. 2009). For the calculation of mean hematology and physiological parameters only animals that were not stressed (332 observations) were considered.

The physiological and blood characteristics evaluated included: rectal temperature (RT), respiratory rate (RR), heart rate (HR), packed cell volume (PCV), total plasma protein (PPT), number of red blood cells (RBC), hemoglobin concentration (HB), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), number of white blood cells (WBC), monocytes (MONO), lymphocytes (LYMP), eosinophils (EOSI), segmented neutrophils (SEGM), platelets (PLAT).

Statistical analysis was performed using SAS® (Statistical Analysis Institute, Cary, North Carolina) with significance level of 5%. The procedures used included averages, confidence interval of 95% (pctlpts command in PROC UNIVARIATE) and frequency (PROC FREQ) to determine the number of observations outside the confidence interval (<2.5% and >97.5%).

Results: Most of the means of the studied parameters were within reference values. However, the values of 2.5 and 97.5 percentiles, which indicate the lower and upper limits, respectively, differed from those previously established in the textbooks. Heart rate was the parameter showing highest divergence, with mean (65.62 beats/minute) and maximum (116 beats/minute) values far above normal range (36-60 beats/minute) for cattle. The mean rectal temperature (39.02°C) was within reference interval (36.7-39.1°C), however the confidence interval (95%) ranged from 37.6 to 41.3 movements/minute and 121 observations were higher than the reference values. Mean respiratory rate (46 movements/minutes) was also within reference interval (26-50 movements/minute), but confidence interval (95%) varied from 40 to 116 movements/minute with 87 observations higher and 25 lower than pre-established values.

Regarding blood parameters, all means were within reference intervals, however there were divergences concerning the upper and lower limits. Mean PCV was of 35.56% with confidence interval of 20.4 - 47.0% and 58 observations were lower and 11 higher than reference interval (24.0-46.0%). The number of red blood cells ranged from 4.43 to 12.15g/dl and 32 observations were higher and 58 lower than reference interval (5.0-10.0g/dl). Thirty five observations of hemoglobin concentration were higher and 41 lower than reference values (8.0-15.0g/dl), with mean of 12.0g/dl. MCHC confidence interval was of 24-45.25% and 192 values were lower and 58 higher than reference interval (30.0-36.0%).

Conclusions: Means of all parameters, with exception of heart rate, were within textbook reference ranges for cattle; however, the upper and lower limits of many variables were different from the reference values, with significant number of observations outside reference interval. Therefore, considering that physiological and blood parameters are essential to evaluate pathological disorders, animal welfare and adaptability to the environment, the use of reference values based on animals subjected to similar environmental conditions is recommended for a correct interpretation of the results.

Animal Welfare

P02-002-018

Risks to cow welfare in an expanding pasture-based dairy industry – veterinary, advisory and farmer perspectives

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Objectives: Cows in pasture-based systems of milk production generally have better welfare than those in confinement systems. However, abolition of the EU milk quota and subsequent intensification in the dairy industry may pose threats to cow welfare in pasture-based systems. ProWelCow (DAFM RSF-14/S/890) is a project which aims to investigate strategies to protect and improve the welfare of Irish dairy cows. This study aimed to identify areas of consensus and disagreement between stakeholders on the main risks to cow welfare associated with herd expansion to better understand the potential role of knowledge transfer in addressing cow welfare issues.

Materials and Methods: A questionnaire of approximately 40 questions was developed, tested and modified accordingly in conjunction with Teagasc dairy research and specialist advisory staff. The survey was conducted in 2015 with dairy farmers (F; n=115) at two national farming events (The National Ploughing Championships and the Moorepark Open Day) and cattle veterinarians (V; n=60) at the Cattle Association of Veterinary Ireland Conference by interview. The survey was distributed amongst Teagasc dairy advisors (A; n=48) at the beginning of an in-service training day where they were asked to complete the survey themselves. The 223 respondents were asked to identify the main welfare issue in Irish dairy cows from the following list: lameness, social stress due to overcrowding (SS), mastitis, metabolic disorders, infectious diseases, poor body condition score (BCS), cold stress and calving difficulties. The results are expressed as a percentage (%) of each group surveyed. A Chi-Square test for equality of two proportions (statistic: X²) was used to investigate whether distributions of response frequencies for the most important cause of poor welfare differed between surveyed groups using PROC FREQ in SAS.

Results: Poor BCS and SS were the 1^o welfare problems according to

22.6% and 14.8% of F, respectively. Of V, 30% selected SS and 28.3% selected lameness as the 1^o welfare issues. SS was selected as the 1^o welfare issue for Irish dairy cows by 46.7% of A and issues around calving by 20% of A. Poor BCS was ranked as the 1^o welfare issue for dairy cows by a higher proportion of F (72.2%) than of V (13.9%) or A (13.9%), ($P < 0.001$). There was good consensus between stakeholders on SS as an important welfare issue with 30.4% of F, 32.1% of V and 37.5% of A reporting it as the 1^o issue ($P > 0.05$). This is supported by the finding that 32.9% of farmers surveyed provided less than one cubicle per cow during the housing period. There was good agreement between F and V as to the importance of lameness to dairy cow welfare (45.5% and 51.5% reported it as the 1^o cause of poor welfare, respectively, $P > 0.05$). However, between the 3 groups, lameness was not recognised as a welfare issue of importance by A (only 3% reported it as the 1^o cause of poor welfare, $P < 0.001$). Both infectious disease and mastitis were more of a cow welfare concern to F (71.4% and 75% reported them as the 1^o cause of poor welfare, respectively) than to V (19.1% and 18.8%, respectively) or A (9.5% and 6.3%, respectively), ($P < 0.01$).

Conclusions: Vets considered lameness as the main welfare issue for cows and poor BCS and infectious diseases of lesser importance compared to farmers. Advisors considered lameness to be a much lower welfare priority than either vets or farmers. These hazard perception gaps indicate a lack of consensus amongst key stakeholders on the primary welfare issues for cows in expanding pasture-based herds. This has implications for the development of strategies to protect dairy cow welfare and suggests that there is a need for better knowledge dissemination on cow welfare issues across, and better communication on the topic between, stakeholder groups.

Animal Welfare

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Association of floor type on health parameters of cattle fed indoors during the finishing phase

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Objectives: The use of indoor confinement concrete slatted floor feeding facilities has grown in the U.S. to comply with increasing environmental regulations and decreasing land availability. Rubber mats manufactured to be installed on top of concrete slats are being promoted to increase comfort and improve welfare and performance. The objective of this project was to evaluate potential health differences associated with various types of slatted flooring in confined beef operations during the finishing phase of production.

Materials and Methods: A cohort study design was conducted using the following groups: Kraiburg slatted flooring mats, Animat rubber flooring, Easy Fix Slat Rubber Solutions for Beef flooring mats, and concrete- only slatted floor pens. For statistical purposes, data was grouped and assessed as mats vs. concrete. Feeder calves were sourced through normal channels by the feedlot's cattle buyer. Calves were individually assessed for general health and existing lameness prior to enrollment. Cattle were evaluated individually and then data were assessed at the pen level. Pen-level morbidity and mortality were assessed. Locomotion scores were assessed at enrollment and within 7 days of slaughter. Descriptive statistics were generated for health and performance outcomes for individual animals and also on a pen basis. ANOVA statistics were used to determine statistical differences

between health and performance parameters between the two groups. Significance was set at 0.05.

Results: Concrete-only pens demonstrated decreased health benefits compared to mat pens. Twelve concrete-only pens and 23 mat pens were included. Compared to cattle on rubber mats, cattle on concrete-only tended to have higher morbidity (17.7% versus 6.6%; $p = 0.07$). When genders were compared within treatment groups, pens of steers on concrete-only pens had higher morbidity (29.7 vs. 10.3%). When gender was examined for mat pens, pens of steers had a 17.8% morbidity rate compared to pens of heifers (5.5%). Pens of cattle on concrete-only slats had 3.1% lameness compared to 1.1% lameness for pens of cattle on mats ($p = 0.02$). When genders were compared between treatment groups, results were very similar (concrete-only: heifers 3.1%, steers 3.2%; mat pens: heifers 1.1%, steers 1.5%). Concrete-only pens also had a higher mortality rate compared to mat pens (1.9% versus 0.7%; $p = 0.03$). Pens of cattle on concrete-only pens had higher locomotion scores compared to pens of cattle on concrete-only (concrete-only 2.1%; mat pens 1.6%).

Conclusions: This study demonstrated increased health benefits for rubber mats. Pens finished on concrete-only slats were more likely to have increased locomotion scores, be identified and pulled for lameness, and have higher mortality. Evaluating the impact of various slatted flooring with health and performance among confined beef cattle in a production setting is important to improve to ensure both the wellbeing of fed cattle and economic viability of cattle feeders. Further work should be done to measure potential differences between mat types as well as economic benefit.

Animal Welfare

P02-002-020

Effects of season of the year on transport-related mortality in fattened cattle

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Objectives: Adverse climatic conditions are strong stressors that animals can face during transport. Ambient temperature affects trailer internal conditions and inadequate microclimate may be one of the largest threats to animal welfare during transportation. Shortcomings in animal welfare during the transportation of cattle may lead to the increased mortality of animals and impaired quality of meat. The aim of this study was to determine the effect of season of the year on the transport-related mortality rate in fattened cattle transported for slaughter in the Czech Republic in the period from 2009 to 2014.

Materials and Methods: Data summarizing the numbers collected from all transports of fattened cattle for slaughter in the Czech Republic in the monitored period were gathered in the database of the Information Centre of the State Veterinary Administration. Seasonal impact, shown by the four seasons: spring (March, April, May), summer (June, July, August), autumn (September, October, November) and winter (December, January, February), on the mortality of cattle during their transport to the processing plants was determined in such a way that for the individual seasons for the whole monitored period, the total numbers of transported cattle and the total numbers of cattle having died were determined and mortality in percentages were calculated for the period from 2009 to 2014. All the data were analysed using the statistical package Unistat v.

6.5. Statistical comparisons between the frequencies of the categorical variables of interest were performed with the Chi-square test (with Yates correction when needed) within the contingency table procedure. Data concerning transport-related mortality rates were compared between the individual seasons monitored in the period from 2009 to 2014.

Results: In the monitored period, in total 631,596 heads of cattle were transported for slaughter, out of which 111 (0.018%) died as a result of this transportation. It follows from our results that season of the year had an important effect on the transport-related mortality rate in fattened cattle. The highest mortality rate (0.032%) was observed in spring months. This was significantly higher ($P < 0.05$) than mortality rate of cattle transported for slaughter in any other season of the year. Lower death losses were associated with transports carried out in summer months (0.018%) and winter months (0.011%). The transport-related mortality rate did not differ significantly in summer and winter. The lowest mortality rate (0.009%) was found in autumn months. The results suggest that it is not extreme ambient temperatures in summer and winter months but rather another factors that are responsible for variability in death losses in cattle transported for slaughter in different seasons of the year. The possible grounds for the highest mortality rate being found in spring months are as follows: reduced quality of feed material, weakened immunity of animals after winter season, increased occurrence of respiratory infections during spring time.

Conclusions: It is obvious from the results that the season of the year plays an important role in terms of animal welfare during the transportation process. As early in course of the route planning, expected effects of the given season should also be taken into consideration.

Animal Welfare

P02-002-021

Case-control study on behaviour of calves related to automatic calf feeding systems after cauterly dehorning with an additional meloxicam treatment

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Objectives: In this case-control study the effect of an additional meloxicam treatment on the behaviour of calves following cauterly dehorning with local anaesthetic and systemic sedation was measured with an automatic feeding systems on commercial dairy farms in the Netherlands. This study was initiated and executed by a veterinary practice in the Netherlands.

Materials and Methods: Five commercial dairy farms were enrolled in this case-control study. All these farms were using the Forster automatic system to feed milk replacer to their calves. In total 54 calves were studied, distributed respectively from farm 1 to farm 5; 12 calves, 24 calves, 4 calves, 9 calves and 5 calves. The dehorning procedure of the calves was done by the same veterinarian throughout the whole study. Calves were first sedated with Xylazine (1ml per calf, Sedamun Dechra), a procainehydrochloride corneal nerve block was given (5ml per horn, Procamidol AST). Calves were blocked by age and randomly assigned to receive a subcutaneously injection of meloxicam (NovemBoehringer Ingelheim, 2.5ml per kg body weight, T-group, 28 animals) or no additional treatment (NOT-group, 26 animals). The meloxicam was given shortly after the procainehydrochloride administration. Treatments were given approximately 5-10 minutes before cauterly dehorning. Only healthy calves without other treatments were enrolled. A continuous observation

was performed by the farmer collecting the data from the automatic feeding system from 2 days before until 3 days after the dehorning. The feeding protocol of the calves on these farms was similar, they were fed milk replacer at a schedule provided by Forster and calves had a fixed limited amount of visits where they could gain milk replacer. Therefore the automatic feeding system measures successful visits when the calves are able to drink milk replacer during 24 hours (MV) and the total visits during 24 hours (TV). The group size of the calves was between 6 and 14 per artificial nipple. Behaviour data was analysed using Excel, with the student T test to determine the difference between treatment groups (p-value

Results: The mean age of the NOT-group was 40.8 days with a standard deviation of 15.2; the mean age of the T-group was 48.5 days with a standard deviation of 20.4 days. There was no statistical difference between the groups (p-value=0.11). The mean TV (total visits during 24 hours) of the NOT-group calves during the period before dehorning (from day -2 until -1) was 35.0 with a standard deviation of 22.0. The mean TV of the T-group calves during the period before dehorning was 35.4 with a standard deviation of 25.6. There was no significant difference between these treatment groups (p-value = 0.93) before dehorning. The mean TV of the NOT-group calves during the period after dehorning (from day 0 until 3) was 31.8 with a standard deviation of 20.7. The mean TV of the T-group calves during the period after dehorning was 38.5 with a standard deviation of 24.5. There was a significant difference between these treatment groups (p-value = 0.02) after dehorning. The mean MV (successful visits when the calves were able to drink milk replacer during 24 hours) of the NOT-group during the whole monitoring period (day -1 until 3) was 4.6 with a standard deviation of 2.5. The mean MV of the T-group in this period was 4.4 with a standard deviation of 2.5. There was no statistically difference in the mean MV between the treatment groups (p-value = 0.21) during this period. This result could be due to the fact that the fixed amount of visits where the calves could drink milk where relatively low to the total amount of visits of the calves.

Conclusions: The additional use of meloxicam after cauterly dehorning had behavioural effects which could be measured with an automatic feeding system. The total visits of the calves after dehorning to the automatic feeding system were positively influenced by the meloxicam treatment. This effect could be explained by a prolonged painless period after the cauterly dehorning procedure. A larger study, with for example webcams added in the protocol, could combine the data of the system with the actual behaviour.

Animal Welfare

P02-002-022

Validation of a novel pedometer algorithm as indicator of animal welfare in a dairy Mediterranean buffalo herd

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Objectives: Change of animal behavior is one of the most important criteria for assessing animal welfare and health. Parameters of animal behavior can be used to build up an early disease warning system aiming to gain a higher health standard. Although this concept is well established

in dairy cows, it is far to be implemented in dairy Mediterranean buffalo (MB). Considering these premises, the aim the current study was to validate in dairy MB a novel algorithm to monitor locomotor behavior based on the output of a 3-dimensional accelerometer, recently established for cows.

Materials and Methods: Fifteen healthy primiparous MBs, kept in a free stall, located in Caserta district, South of Italy were enrolled. Data regarding, standing, walking and lying activities were collected by mean of continuous videotaping (HD-Digital System, Conceptronic, Germany; gold standard), and simultaneous recording of locomotion with 3-direction accelerometers, attached to the metatarsus of one hind limb (RumiWatch®, ITIN+HOCH GmbH, Fütterungstechnik, Liestal, Switzerland). Validation of the algorithm was divided into 2 major experiments. Experiment 1 was performed to validate dichotomic (i.e. stand-up, lie-down, number of lying bouts) and continuous output data (i.e. lying time), using a data acquisition period of 24h. Experiment 2 was performed to validate the number of strides. The latters originated from 3 data acquisition periods (video recording and pedometer) of continuous non-lying activity of 20 minutes each one. Data obtained from the gold standard and the pedometers were compared, calculating proportions (dichotomous data) or the mean relative measurement error (RME) (continuous data) according to the following formula: percent deviation = $(100/\text{video-recording observation}) \times \text{absolute value (video-recording observations - pedometer observations)}$. RME <1% was rated very low, 1 to 5% was rated low, and 6 to 20% was rated moderate. Agreement between continuous variables was expressed by means of Spearman nonparametric correlation coefficient (r_s). All statistical analyses were undertaken using SPSS (Version 17.0, SPSS, Chicago, IL).

Results: In experiment 1, all stand-up ($n = 110$) and lie-down events ($n = 110$) and all lying bouts ($n = 113$) were correctly detected, while only 2 out of 224 walking bouts (0.89%) were not recorded by the algorithm. A very low mean RME associated with the estimated lying time (546.29 min/animal, mean value) was detected (0.13%), with a very high correlation compared with the video recordings ($r_s = 1$, $P < 0.0001$). In experiment 2, the median number of strides per 60 min of recording time was 67 (range: 46–116 strides), with a mean RME of 8.61%, and a very high correlation of $r_s = 0.957$ ($P < 0.0001$) between video recordings and algorithm output was estimated.

Conclusions: Health monitoring by means of an automated livestock management system was performed for the first time in MB. The encouraging preliminary results suggest that the novel pedometer may detect several locomotion behaviors in these ruminants with a high accuracy. The differences detected in the walking activity can be related to a specific scratching behavior present in MB but not found in cattle and misclassified by the software. Further investigations are warranted to eliminate this error and validate additional parameters.

Objectives: The purpose of this study was to compare a one-time oral administration of four non-steroidal anti-inflammatory drugs in their ability to control the pain and distress associated with cautery dehorning in dairy calves. The NSAIDs investigated have pharmacokinetic properties in cattle that produce a persistent plasma concentration, which may allow for prolonged analgesia with the added practicality of a simple administration regimen. All drugs were administered orally at the same dose on a mg/kg basis.

Materials and Methods: One hundred and eighty five calves aged approximately 6 to 9 weeks old were either sham dehorned ($n=31$) or cautery dehorned following oral administration of carprofen ($n=31$), firocoxib ($n=31$), flunixin meglumine ($n=30$), meloxicam ($n=31$) or a placebo in randomized, controlled trial. A standard dose of 2.0 mg/kg was given to all calves receiving an oral NSAID. In addition, all calves received local anesthesia prior to actual or sham dehorning. Cortisol concentrations, heart rate, mechanical nociception thresholds, ocular and dehorning area temperatures and average daily gains were evaluated. A linear mixed effects model with repeated measures was used for statistical analysis. Planned contrasts evaluating analgesic treated calves and placebo treated controls were also analyzed.

Results: Analgesic administration was associated with changes in ocular and dehorning area temperature differences ($P=0.020$). In addition, administration of oral meloxicam, flunixin meglumine, and firocoxib resulted in decreased cortisol concentrations compared to placebo treated controls for the first 24 h post-dehorning (AUROC-24) ($P=0.0499$). Moreover, meloxicam attenuated the maximum cortisol released compared to placebo treated calves ($P=0.018$). Although carprofen administration reduced heart rates after 24 h ($P=0.0466$), it was unable to control the acute stress associated with cautery dehorning during the initial 24 h. Although a treatment effect ($P < 0.0001$) was observed in the determination of mechanical nociception threshold among all treatment groups, no differences were observed among treatment groups dehorned. Average daily gain was not affected by treatment ($P=0.91$).

Conclusions: A one-time dose of 2.0 mg/kg of oral meloxicam, flunixin meglumine, or firocoxib reduced the acute stress response associated with cautery dehorning; however, carprofen administration was associated with increased cortisol concentrations and dehorning area temperatures for the initial 24 h. Given the reduction in maximum cortisol concentrations, meloxicam appears to be the most potent NSAID evaluated.

Animal Welfare

P02-002-023

A Field Trial Comparing Four Oral Non-Steroidal Anti-Inflammatory Drugs On Controlling Pain And Stress Associated With Cautery Dehorning In Calves

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Animal Welfare

P02-002-024

Heart rate and cardiac vagal tone of Holstein–Friesian dairy cows with assisted calvings

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Objectives: Besides several aspects of management, giving assistance in calving has a great importance. Careful assistance can help to minimize unnecessary pain and distress during calving; however, care must also be taken when deciding on the necessity and timing of assistance during calving. In the present work, we were interested in whether the timing and technicality of obstetrical intervention (early and unnecessary vs. professional) and the management of parturition (individual vs. group calving) has an impact on cow welfare during calving in the early postpartum.

Materials and Methods: Heart rate (HR) and the high frequency (HF) parameter of heart rate variability were calculated, the latter is highly sensitive for alterations in cardiac vagal tone. We divided calvings into four groups as follows: 1) unassisted calving in the individual pen (UCI, n = 21); 2) unassisted calving in the group pen (UCG, n = 19); 3) assisted calving with professional obstetric intervention (ACP, n = 20); 4) assisted calving with inefficient obstetric intervention (ACIN, n = 19). HRV analysis was performed for the following periods of the peripartum: 1) prepartum period (PREPART) between 96 h before the onset calving restlessness and; 2) parturition (PART) (between calving restlessness and the time of birth); and 3) postpartum period (POSTPART) between birth and 48 h after calving. HR and HF parameters were calculated as areas under the curves (AUC). Behavior was recorded with a closed-circuit camera system including two day/night outdoor cameras installed above the pen allowing subsequent matching of the stages of calving and HR recordings.

Results: No difference was found between calving groups in areas under the HR and HF curves during PREPART. Heart rate AUC_{PART} was similar across UCG and ACP groups (P = 0.875), but it was greater in these groups than in UCI cows (P = 0.008 and P = 0.033, respectively). Heart rate AUC_{PART} was greater in ACIN animals compared to any other calving groups (P < 0.001 for all comparisons). AUC_{POST} differed not across UCI, UCG and ACP groups (P = 1.000 for all comparisons), but it was greater in UCIN cows than in animals from UCI, UCG and ACP groups (P = 0.022, P = 0.001 and P = 0.014, respectively). HF AUC_{PART} was similar across UCG and ACP groups (P = 0.386), and across UCI and ACP (P = 0.299) as well, however, it was greater in UCG cows compared to UCI cows (P = 0.011) reflecting on higher parasympathetic tone of animals calved in group. HF AUC_{PART} was lower in ACIN cows compared to the other calving groups. Area under the HF_{POST} curve was similar across UCI, UCG and ACP groups (P = 1.000 for all comparison), but it was lower in UCIN cows than in animals from UCI, UCG and ACP groups (P = 0.006, P = 0.005 and P = 0.001, respectively). Cows from UCI, UCG and ACP groups showed a similar parasympathetic activity within POSTPART (P = 1.000 for all comparison), while vagal tone had the lowest peak in ACIN cows as reflected by HF minimum (P < 0.001 in all comparisons). Similar to HR, HF required shorter time to return baseline levels in UCI, ACP and ACIN groups than in UCG cows (P < 0.001 for all comparisons) and time to return to baseline was similar between the former groups.

Conclusions: Heart rate and cardiac parasympathetic activity reflects on a higher stress load on ACIN cows compared to the other groups during parturition and in the early postpartum. Our data demonstrate the impact of appropriate timing and technicality of obstetrical intervention on cattle welfare in cases of assisted calvings. Based on the present

findings, physiological indicators of stress, such as HRV particularly during the peripartal period may reveal opportunities for improvements in calving management in the future.

Animal Welfare

P02-002-025

Cortisol concentrations, heart rate and heart rate variability of dairy cows with different temperament during and after transrectal examination

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Objectives: Hypothalamic–pituitary–adrenal (HPA) axis and cardiac autonomic nervous system (ANS) activity was evaluated in response to transrectal examination in non-lactating Holstein–Friesian cows with different temperament. The main objective of our study was to determinate the differences in pain-related behaviors, plasma and salivary cortisol concentrations, heart rate (HR) and heart rate variability (HRV) related to behavioral characters before, during and following transrectal examination.

Materials and Methods: Frequency of behaviors (restlessness, step/kick behavior and urination/defecation/vocalization) performed during fixing the heart rate monitors were used to classify cow temperament as Low Responder [consisted of 20 cows with the lowest reactivity (n = 20)] or High Responder [the 20 cows with the highest reactivity (n = 20) to the procedure]. The measurement period lasted from the last 5 min before the examination until 120 min after the procedure has finished. Activity of the autonomic nervous system was assessed by HR and HRV parameters. Blood and saliva samples were collected at 5 min before (baseline) and 0, 5, 10, 15, 20, 30, 40, 60 and 120 min after the examination has finished to determine cortisol concentrations. Cardiac parameters included HR, the root mean square of successive differences (RMSSD) between the consecutive interbeat-intervals, the high frequency (HF) component of HRV and the ratio between the low frequency (LF) and HF parameter (LF/HF ratio).

Results: Baseline levels of plasma and saliva cortisol did not differ between groups. Following the examination, peak plasma and saliva cortisol levels (P = 0.002 and P = 0.001, respectively) and the amplitude of the plasma and saliva cortisol response (P = 0.001 and P = 0.003, respectively) were higher in High Responder cows than in Low Responders. Areas under the plasma (P = 0.020) and saliva cortisol response curves (P = 0.037) were greater in High Responder cows. Plasma and salivary cortisol levels correlated significantly at baseline (r = 0.91, n = 40, P = 0.001), right after examination (r = 0.98, n = 40, P = 0.001) and at peak levels (r = 0.96, n = 40, P = 0.001). Basal heart rate was higher in High Responder cows than in Low Responder cows (P = 0.017). Area under the heart rate response curve was higher in Low Responder cows than in High Responders (P = 0.033), however, maximum heart rate and the amplitude of the heart rate response showed no differences between groups. Baseline values of RMSSD and HF did not differ between groups. Minimum values of both parameters calculated for the 5-min transrectal examination were

higher in High Responder cows. Following the examination, response parameters of RMSSD and HF did not differ between groups. Except from baseline values, the maximum ($P = 0.004$) and the amplitude of LF/HF ratio response ($P = 0.001$) and area under the LF/HF response curve ($P = 0.022$) were lower in Low Responder cows compared to High Responders suggesting lower sympathetic activation of the ANS in Low Responder animals.

Conclusions: Our results demonstrate that cortisol concentrations and parameters of HRV may complement each other, providing a reliable option for the assessment of acute pain in dairy cattle. Animals with an excitable temperament exhibit increased plasma and salivary cortisol concentrations and higher cardiac autonomic responsiveness to transrectal examination than calmer cows. Salivary cortisol could substitute for plasma cortisol when assessing response of cattle to severe stress.

Animal Welfare

P02-002-026

Welfare parameters in dairy cows reared in tie-stall and open-stall farming systems

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Objectives: Housing and management conditions are expected to affect dairy cattle welfare. The animals could experience pain or distress as a result of widely accepted management practices, and possibly suffer being kept under apparently "unnatural" conditions. The animal welfare assessment requires the use of multiple indicators in order to analyse the heterogeneity of aspects involved. The aim of this study was to compare animal welfare of dairy cows reared in tie-stall and open-stall system by means of metabolic, immunological and stress related parameters.

Materials and Methods: The study involved 80 pluriparous lactating cows belonging eight dairy farms located in the area of Mugello (Florence district) reared in different system: tie-stall (TS) and open-stall (OS). Ten blood samples were collected in the morning from the jugular vein using vacutainer tubes containing EDTA to estimate non esterified fatty acids (NEFA), total proteins (TP), creatinine (Creat), nitrogen ureic (BUN), betahidroxiacetato (BHBA), aspartate-aminotransferase (AST) alanina-aminotransferase (ALT), calcium (Ca), chloride (Cl), potassium (K), oxygen free radicals (OFR), haptoglobin (HP), serum lisozyme (SL), bactericidal activity (SBA) and hair cortisol. Hair samples were carefully cut from the tail switch using clippers and were frozen at the temperature of -20°C ; cortisol was analysed by the method described by Accorsi et al. At the same time body condition score (BCS) was recorded. The experiment was performed in accordance with European Commission regulations (86/609/EEC) and Italian law (116/92).

Results: Results showed that the housing system affected some parameters such as ALT, AST, N ureic, BHBA, OFR and cortisol. Most of these showed mean values within the range of reference without revealing signs of suffering. An interesting outcome regarded OFR level that resulted higher in the OS system likely as consequence of a higher productive effort. Results regarding hair cortisol were particularly interesting because it is known as an indicator of chronic stress: TS

displayed significant ($P < 0.001$) higher mean value than OS (2.8 ± 1.43 vs 1.6 ± 1.05 pg/mg respectively). The two housing systems did not differ for BCS, that was 3.1 and 3.0 respectively for both the solution.

Conclusions: In this study did not emerge a situation of evident suffering in animals reared in TS. Tethered cows may have an improved welfare quality if they benefit from comfortable and clean stalls, quantitatively and qualitatively adequate feeding and watering, access to exercise and not in the least a good relationship with the stockperson.

Animal Welfare

P02-002-027

Higher animal welfare systems: The business case for beef cattle

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Objectives: Food businesses struggle to find higher animal welfare standards that are applicable to different parts of the globe. Even when working in countries with a good animal welfare (AW) assurance schemes' penetration, it is very challenging to find AW assured beef cattle. To overcome this challenge, food companies perform their own audits or questionnaires on the different suppliers. In order to support those companies to find beef suppliers that operate under higher animal welfare conditions, the Food Business Programme at CIWF has produce different resources, being one of them the animal welfare potential matrix.

Materials and Methods: The animal welfare potential matrix is a summary table which outlines the welfare potential by production system using a 'Bad-Better-Best' model, showing a summary version of the different systems that companies can find in their supply chain and can be consider better or best in terms of animal welfare. To define the bad, better and best systems in beef production and to make sure they were globally relevant, commercially viable and sustainable, different experts around the world were consulted, as well as different companies that are already working with higher animal welfare cattle in their supply chains. Also, to make sure the document was science based, a literature review of the main welfare issues in beef cattle was made. The structure of the document followed previous ones from other species, taking into account the three components that generate quality of life to the animals: mental wellbeing, physical wellbeing and expression of natural behaviour. The better model for beef cattle, which is the most commonly used by companies as a reference of a commercially viable higher Animal Welfare system, features a semi-extensive system that is pasture reared during grazing season and higher animal welfare housing when off pasture.

Results: The matrix is now being used by different companies to characterise current suppliers and look for prospect ones; to feed into animal welfare policies and as a support document to create questionnaires for internal and own audits to the suppliers. The first results show a tendency from companies to start to source their beef cattle from countries that are able to produce pasture based beef cattle (even if just for a limited part of the year). One interesting finding related to pasture based systems was that food businesses' interest shown on pasture is not just based on an animal welfare component, but is also based on a holistic approach to sustainability, including economic volatility, environmental impact, low risk investment and as a point of differentiation in parts of the world where the meat consumption has reached a plateau and will probably decrease. Finally, it was reported by

different companies that there are still major difficulties to find suppliers housing beef cattle housed in higher animal welfare conditions, mainly due to the fact that this side of the production is not as valued and recognised as the pasture use.

Conclusions: The beef welfare potential matrix features better and best models of animal welfare that are commercially viable at a global level. It has shown that there is a business case for beef sourced from higher animal welfare systems that include pasture based systems as well as higher animal welfare housing.

Animal Welfare

P02-002-028

Thermographic images for heat tolerance evaluation in Curraleiro Pe-Duro, Pantaneiro and Nelore cattle

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Objectives: Adaptability evaluation and heat tolerance of the animals are mainly determined by the physiological parameters such as respiratory rate and body temperature. However, new tools, such as infrared thermography, are alternatives to assess the impact of environmental factors on thermal stress in animals. Therefore, the objective of this study was to compare physiological and thermographic responses to heat stress in three breeds of cattle.

Materials and Methods: Forty-five males of the Pantaneiro, Curraleiro Pe-duro and Nelore breeds (15 animals each), approximately three years of age were used. The animals were reared as one group with the same environmental and treatment conditions and were fed with a 70% roughage and 30% concentrate diet on a dry matter basis. For the duration of the experiment, animals were maintained in a roofless corral with lateral wooden slating. Before physiological collections, the animals were maintained in an open corral for at least two hours without shade and contained in a chute. The physiological parameters measured included: respiratory rate, heart rate and rectal temperature, evaluated during the morning at 8:30h, and in the afternoon, at 15:30h, with six repetitions. Animal surface and ground temperatures were obtained using an infrared camera FLIR® T400. Two images were taken from each animal, at a distance of 1.5m, one laterally of the whole body and the other of the head region. For this, the animals were taken calmly to a covered area immediately after physiological measurements, so no time was allowed for the animals to adapt to the shaded area before recordings were completed. Standard Quickreport® tools were used for analysis of the images. Air temperature, wind speed, relative humidity were obtained from a mobile weather station and used to calculate the temperature and humidity index (THI). The statistics analysis included an analyses of variance, correlation, principal factors, as well as canonical analyses.

Results: Breed affected all physiological parameters and all surface temperatures ($P<0.05$). Nelore breed had the lowest heart rate (103.91 mov/min). The Curraleiro Pe-duro breed had the lowest body area (35.34°C), neck (35.36°C), rump (35.14°C), head (35.38°C), groin (34.79°C) and axilla (35.14°C) temperatures as well as rectal temperature (38.63°C) and the highest respiratory rate (46.55 breaths/min). The period of the day influenced the surface temperatures, as well

as respiratory and heart rate of the animals ($P<0.05$). In the morning period, the Curraleiro Pe-Duro breed showed the lowest values of body area (32.87°C), neck (33.22°C), rump (31.77°C), muzzle (29.07°C), head (31.64°C), groin (32.22°C) and axilla (32.74°C) temperatures, compared to the other breeds. In the afternoon period, little difference in the surface temperatures was observed among the breeds studied. For the physiological parameters, the Nelore breed showed the lowest heart rate, both in the morning and in the afternoon, as well as the higher rectal temperature and the lowest respiration rate, in the afternoon period. For the Curraleiro Pe-Duro breed, the respiratory rate was higher, especially in the afternoon. The correlations between the surface and the environment temperatures were high and positive. Rectal temperature showed a low correlation with respiratory rate (0.21) but was not correlated with heart rate (0.05). The principal component analysis showed that 76% of the variation was explained by the surface temperature, physiological and environmental parameters evaluated.

Conclusions: The Curraleiro Pe-Duro breed had the lowest rectal and surface temperatures independent of the period evaluated, with fewer animals that suffered with the climatic conditions, so this may be considered the best adapted when heat challenged under the experimental conditions. Thermography data showed a good correlation with the physiological indexes, and body area, neck and rump were the main points.

Animal Welfare

P02-002-029

Heat stress response in zebu cattle using physiological traits and thermographic images

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Objectives: As heat stress can affect the productive and reproductive life of beef cattle, this study was carried out to evaluate the heat tolerance of five zebu cattle breeds using physical, physiological and hematological traits as well as thermographic responses.

Materials and Methods: Forty cows of the Gir, Girolando, Nelore, Sindhi and Indubrasil breeds (eight cows each), approximately three years of age, non-pregnant and non-lactating were evaluated. Body weight, withers and hump heights as well as thoracic circumference were recorded using a measuring tape. The density and length of the hair as well as skin color and thickness were evaluated. The physiological parameters measured included: respiratory rate, heart rate and rectal temperature, evaluated during the morning and in the afternoon. Blood samples were collected for hematological evaluation. Animal surface and ground temperatures were obtained using an infrared camera FLIR® T400. Two images were taken from each animal, at a distance of 1.5m, one laterally of the whole body and the other of the head region. Standard Quickreport® tools were used for analysis of the images. Red and white cell number, platelets and hemoglobin concentration were obtained using an automatic cells counter. Monocytes, lymphocytes, segmented neutrophils and eosinophils numbers were obtained by counting one hundred cells under a microscope. Total plasma protein concentration was determined by refractometer. Air temperature, wind speed, relative humidity were obtained from a mobile weather station and used to calculate the temperature and humidity index (THI). The statistics analysis included an analyses of variance, principal factors, as well as logistic regression and calculation of odds ratio.

Results: Breed influenced all the physical traits ($P < 0.05$). Indubrasil and Girolando animals had the highest body weight and thoracic circumference (559.75 and 552.12 kg and 2.05 and 2.05 cm, respectively) however, Girolando and Nelore animals had highest withers height (1.40 and 1.39m, respectively). There were significant differences in the rectal temperature, heart and respiratory rates between breeds ($P < 0.05$). Gir and Indubrasil breeds had the highest rectal temperatures (39.05 and 39.00°C, respectively). Breed was significant for surface temperatures ($P < 0.05$). Eye and brain surface temperatures were the most affected by environmental parameters. Also, environmental parameters affected packed cell volume and red cell number. Nelore animals had the lowest packed cell volume (37.46) and Nelore and Indubrasil animals had the lowest value of total plasma protein (7.58 and 7.46, respectively). The principal component analysis showed that in the morning, 63.13% of the variation was explained by the physiological and physical variables of the animals. Odds ratio test showed that the Gir breed was three times more likely to have higher rectal temperature compared with Sindhi as confirmed by the logistic regression. When the black globe temperature approached 35°C, the probability of the Gir animals having rectal temperatures above normal was approximately 70%.

Conclusions: Physical characteristics were important in assessing the physiological response when animals were heat challenged, but these characteristics alone are not responsible for conferring tolerance to heat, which also included a set of morphological characteristics and physiological adjustments. Gir animals were the least adapted to the climatic conditions of the experiment and Girolando and Sindhi breeds showed the best physiological indices when heat challenged. Thermography showed a high correlation with the physiological indexes, especially with the rectal temperature and surface temperatures and axilla, neck and eye were the main points.

Animal Welfare

P02-002-030

Effect of space allowance and floor type on intake, performance, hoof condition and blood physiological variables of finishing beef heifers

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Objectives: Previous studies have shown that increasing space allowance above 2.0m² for heifers (Fisher et al., 1997) and 3.0m² for steers (Hickey et al., 2003) has no benefit on animal health or welfare. There have been suggestions from advocacy groups in the EU to increase the space allowance for finishing cattle above 3m² per animal although there is no scientific evidence to support this change. Therefore, the objective of this study was to investigate the effect of space allowance (3.0, 4.5 and 6.0 m²) on fully slatted concrete floors and 6.0 m² on straw on intake, performance, hoof health and blood physiological variables of finishing beef heifers.

Materials and Methods: Two-hundred and forty crossbred beef heifers, (mean initial liveweight, 505 (SD 35) kg) were blocked by breed, live weight and age and, from within block, were randomly assigned to one of four treatments; 3.0, 4.5 and 6.0 m² on slatted concrete floors and 6.0 m²

on straw. For each treatment there were six pens of ten animals. They were offered a maize silage and rolled barley based total mixed ration to appetite. Feed was weighed in daily and refusals were measured twice weekly. Heifers were weighed before assignment to treatment (day (d) 0) and at 21 d intervals until slaughter (d 105). Hooves were inspected for lesions on d 0 and d 105. Jugular blood samples (K₃EDTA (1×6 ml) and no-anticoagulant (1× 8.5ml)) were collected at 21 d intervals. Whole blood samples (K₃EDTA) were analysed for haematological profiles (white blood cell, red blood cell numbers and haemoglobin concentrations) using an ADVIA haematology analyser. Serum samples were assayed for metabolite concentrations (total protein, albumin, globulin, non-esterified fatty acids, creatine kinase and beta-hydroxybutyrate) using an Olympus Instrument. Post-slaughter, carcass weight, kill-out proportion, hide weight and carcass conformation and fat score (1-15) was determined. Statistical analysis was done using SAS (version 9.3; SAS Institute, Inc.). The model included the fixed effect of treatment and batch and the random effect of block. Pen was the experimental unit for intake and FCR and animal was the experimental unit for all remaining variables. Haematological data was analysed using repeated measures ANOVA.

Results: There was no effect ($P > 0.10$) of space allowance or floor type on DMI. Space allowance had no effect on ADG or FCR ($P > 0.10$); however ADG was higher (1.34 v. 1.19 kg) and FCR was better (8.42 v. 9.45 kg) for heifers on straw compared to those on slats at 6.0m² ($P < 0.01$). There was no difference in slaughter weight, carcass weight, kill-out proportion, hide weight and carcass conformation score or fat score among treatments ($P > 0.10$). Space allowance and floor type had no effect ($P > 0.10$) on the number of hoof lesions, blood metabolite concentrations or any of the haematological variables.

Conclusions: Results showed that increasing space allowance above 3.0m² for finishing beef heifers on fully slatted concrete floors did not affect animal intake and performance. At the same space allowance (6.0m²), housing heifers on straw as opposed to concrete slats increased ADG but this did not result in a significantly heavier slaughter or carcass weight. Space allowance and floor type had no effect on hoof health or blood physiological variables.

Animal Welfare

P02-002-031

White Blood Cell Count After Disbudding in Calves Treated with Low Level Laser Therapy

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Objectives: We aimed to investigate the effect of different low level laser therapies (LLL), i.e. red laser and infrared laser, commonly known as cold laser, in the white blood cell count (WBCC) of disbudded calves, as a novel and alternative treatments for wound healing, improving animal welfare by lowering recovery time and decreasing inflammation process.

Materials and Methods: Twelve male Holstein calves, four weeks old, disbudded by hot iron were used in the study. Local anesthesia was performed with lidocaine hydrochloride 2%. The cornual branch of the zygomaticotemporal nerve was blocked associated with infiltrative anesthesia around the cornual button. After hair clipping and antiseptic

with 2% chlorhexidine, bilateral cauterization of germinal buds was made. For pain relief after disbudding, we administered metamizol sodic (25mg/kg, SID) during three days.

The animals were divided into three different treatment groups:

- 1) control (ointment with zinc oxide) - 4 calves;
- 2) LLLT with red laser ($\lambda = 660 \text{ nm}$, 100mW, 2J per point) - 4 calves;
- 3) LLLT with infrared laser ($\lambda = 808 \text{ nm}$, 100mW, 2 J per point) - 4 calves.

Only one treatment with zinc oxide ointment, red laser and infrared laser was made, according to the described groups, immediately after hot iron disbudding.

Blood samples were collected for WBCC evaluation in seven different times: 1h before disbudding; 2h and 4h after disbudding; 3, 7, 14 and 21 days after disbudding.

Statistical analysis was made with Tukey's test ($p < 0.05$) using Minitab Statistical Software.

Results: We compared the total leukocyte count among the different treatments within the same time. According to our results, regardless of the group, statistical analysis showed no significant difference in any of the studied periods. There was no significant difference among the treatments in the total number of neutrophils, eosinophils, basophils, lymphocytes and monocytes within the same time. When the comparisons were made among the different times but within the same group, there was no difference as well.

Local burn injuries could lead to systemic alterations in WBCC if secondary infections and/or severe inflammation were present, but, in our study, there were no animals with systemic responses to them. Therefore, no alterations in WBCC were found regardless to the treatment group.

Conclusions: We concluded that there was no difference in the WBCC in hot-iron disbudded calves, regardless the treatment group. Therefore, the WBCC did not present itself as an accurate indicator for acute inflammation in disbudded calves when local burn injuries remain without complications.

The advantages of LLLT as a practical and effective treatment for wound healing after hot-iron disbudding in calves need to be addressed with more studies concerning novelties for an old practice, improving the animal welfare after such procedure.

Animal Welfare

P02-002-032

Validation of the algometer for measuring udder discomfort in postpartum lactating dairy cows

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Objectives: With modern milk production, we may hypothesize that high-producing cows possibly experience some level of discomfort due to udder distension. The pressure algometer has been used to evaluate pain but it was never validated. A pressure (in kg of force, kgf) is exerted

using the device in the body region of interest until the animal responds with an avoidance reaction. Objectives of the current study were (1) to quantify the precision of the measurement when collected multiple times (within and between observer repeatability), and (2) to evaluate whether such a measure would actually represent a valid measure of udder discomfort.

Materials and Methods: 1) Repeatability: algometer measurements were obtained from three herds. In herds A (n=30) and B (n=14) measurements were obtained once a week just before milking during the first two weeks of lactation. In herd C (n=36), measurements were obtained prior to milking, during five consecutive days. Measurements were obtained twice by two operators. The concordance correlation coefficient (CCC) and the limits of agreement plot were used to investigate within and between observer repeatability.

2) Validity: two experiments were conducted. In experiment 1 (herds A and B) cows were randomly selected to be milked incompletely (10-14l of milk/day; n=21) during the first five days in milk or to be milked completely (n=19). Algometer measurements were collected once on each cow during the treatment period. The pressure needed to trigger an avoidance response was then used as outcome variable in a linear regression model with treatment group as the main predictor, and with herd as fixed effect. The hypothesis was that incompletely milked cows would respond more readily to pressure. In experiment 2 (herd C, n=36) measures were collected five times a day during five days: immediately after morning milking (MM; time 0), +4h, +5h30, +7h30 and +8h30 following morning milking. Measurement obtained was used as outcome variable in a linear mixed regression model with time since MM as main predictor, and with day of measurement and cow as random effects. The hypothesis was that cows would respond more readily to pressure with increasing udder filling. A null model was evaluated for variance partitioning.

Results: Algometer measurements were quite repeatable with good within observer agreement (CCC = 0.66; 95% CI [0.57, 0.74]) and moderate between observers agreement (CCC = 0.52; 95% CI [0.38, 0.63]). In experiment 1, mean avoidance response of 6.4 kgf and 5.3 kgf was observed in cows incompletely and completely milked, respectively. Treatment group was not a significant predictor of the cow response to pressure (P=0.22). These results indicate that either the incomplete milking did not cause measurable levels of udder discomfort, or the algometer was not a good device to measure the discomfort. In experiment 2, variation in the algometer response was mostly due to time of the day (51%) and to the cow (40%), while only a small variation was due to the day of the measurement (8%). Response to pressure varied significantly with number of hours after milking (P<0.01); further, parity of the cow significantly affected that relationship. Amount of pressure needed to trigger a response, however, did not decrease with increasing udder repletion (i.e. with no. of hours since milking). Again results may either indicate that udder repletion does not cause discomfort or that algometer is not a good device to measure discomfort associated with udder distension.

Conclusions: This was the first study reporting repeatability of the pressure algometer for measuring udder discomfort and it showed good repeatability. We could not confirm that the algometer provide a valid measure of udder discomfort. Cows' response to the pressure algometer was highly influenced by time of the day at which the measurement was taken and by cows' characteristics.

Animal Welfare

P02-002-033

Effects of Transdermal Flunixin Meglumine on Pain Biomarkers at Dehorning

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Objectives: The objective of this study was to evaluate the analgesic effects of topical flunixin meglumine (Finadyne Transdermal; MDS Animal Health, UK) when given at the time of dehorning on pain associated biomarkers.

Materials and Methods: Twenty four weaned male Holstein calves, ages 6 to 8 weeks of age were enrolled into the study. The calves were randomly assigned to one of three treatment groups of: 1) topical flunixin and dehorn (DH-FLU); 2) topical flunixin and sham dehorn (SHAM-FLU); and 3) placebo and dehorn (DH-PLBO). Treated calves had topical flunixin meglumine applied at the label dose of 3.33 mg/kg concurrently with dehorning. Untreated calves had a placebo which matched the drug's color, odor, and viscosity applied at the time of dehorning. The volume of placebo applied was equal to the calculated dose of the drug in milliliters. The dehorning procedure consisted of applying an electrocautery dehorner to the horn tissue for 10 seconds. A second identical dehorner that was not heated was used for the sham dehorning. The sham procedure consisted of applying the dehorner to the horn for 10 seconds. Biomarker parameters collected and analyzed included: infrared thermography (IRT), mechanical nociception threshold (MNT), plasma cortisol, and Substance P. Infrared thermography images for maximum temperature were analyzed using commercially available research software. Plasma cortisol and Substance P concentrations were determined by radioimmunoassay.

Results: There were no differences in the maximum temperatures (°C) detected for the IRT measurements of the medial canthus of the eye, dehorning site and adjacent skin for the DH groups. Mean control point MNT measurements at 49 hours were 3.14 kgF, 3.46 kgF and 1.43 kgF for the DH-FLU, Sham-FLU and DH-PLBO groups respectively (P = 0.0001). No other differences of MNT were detected between groups for the other test sites and time points.

Peak plasma cortisol concentrations were reached at 20 minutes post dehorning for the DH-FLU and DH-PLBO groups and 10 minutes for SHAM-FLU group. Peak plasma cortisol concentrations were 32.0 ng/ml, 12.7 ng/ml, and 28.8 ng/ml for the DH-FLU, SHAM-FLU and DH-PLBO groups respectively. Cortisol concentrations were statistically lower for the DH-FLU group at 90 minutes post dehorning compared to the SHAM-FLU and DH-FLU group (P = 0.04). At 4 hours post dehorning, the concentrations were 6.36 ng/ml, 11.91 ng/ml, and 8.94 ng/ml (P = 0.1142) for the DH-FLU, SHAM-FLU, and DH-PLBO groups.

Substance P concentrations at 2 hours post dehorning were 27.16 pg/ml, 26.04 pg/ml, and 33.09 pg/ml for the DH-FLU, SHAM-FLU, and DH-PLBO groups respectively (P = 0.4577). Substance P concentrations were 27.81 pg/ml, 27.08 pg/ml, and 30.36 pg/ml for the DH-FLU, SHAM-FLU, and DH-PLBO groups respectively (P = 0.8496) at 4 hours post dehorning. No differences in Substance P concentrations between groups were detected for all time points.

Conclusions: Based on the findings presented here flunixin meglumine, when given as a topical solution, does not have significant effects on biomarkers associated with pain. Other NSAIDs, like meloxicam, have been tested to be efficacious at the time of dehorning and may be more appropriate for use.

Animal Welfare

P02-002-034

Education of the Dairy Employee on Animal Care – A Practical Approach using Dairy CARE 365

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Objectives: The dramatic increase in the size of US dairy operations in the last two decades changed the dairy employee constituent from family based and local hires to many employees from other countries with a language barrier and no farm animal experience. The goal of Dairy CARE 365 is to illustrate to the consumer that the dairy community is concerned with the welfare of the animals entrusted to their care. The focus of the program has been to educate employees in the area of cattle care and handling, as they are in daily contact with the animals. This education may have a positive impact on both the welfare of the employee and the dairy cow.

Materials and Methods: There are two types of materials that constitute the educational content of the Dairy CARE 365 program. Firstly, and the core of the program, are the training Modules containing self-advancing slides with embedded videos to illustrate proper techniques and procedures. Approximately twenty minutes in length, they are narrated in English and Spanish, with an assessment at the conclusion, also narrated, with a traveling arrow over each response, if lack of literacy is an issue. With successful completion, a certificate is generated for the employee. At present there are seven modules available with topics including low stress cattle handling, euthanasia, treatment of the non-ambulatory cow, and care of the new born calf. The modules were developed through a collaborative effort utilizing several veterinarians with expertise and training in the topic area covered. The second set of materials consists of SOP's (Standard Operation Procedures) in WORD format, with boiler plate language, that is designed to be customized to their specific farm. Through these materials, it is possible to implement this program at a group meeting, and then owners and employees can return to their farm and devise their individual SOP's and utilize the Modules to train employees in these specific areas. The workshops usually consume three hours and we discuss two important SOP's, construct an Animal Care Commitment for each farm, and show a training Module to conclude.

Results: This effort has been embraced by the both the veterinary profession and the dairy community. It has been a great vehicle for veterinarians to facilitate training and aid in SOP development, inserting their expertise into the center of animal care on the dairies they serve. Many of the US milk processing plants are asking their patrons, the farms selling them milk, to attend these workshops. NMPF (National Milk Producers Federation) has partnered with Dairy CARE to utilize these training materials to augment the FARM (Farmers Assuring Responsible Management) program. To date we have distributed, from direct requests, more than 2,500 copies of the Modules, 780 registrants for modules from our website, and they are in 30 countries and translated into 6 different languages. More than 50 Dairy CARE training sessions have been held in 21 States in US, as well as Canada, at the behest of 15 processors (representing more than half of the US dairy herd) and several veterinary clinics. There have been over 1,000 attendees at these workshops representing more than 2 million dairy cows and over 10,000 dairy employees. Finally, other allied industries, such as nutrition

and breeding companies, have utilized these materials to better serve their dairy clients

Conclusions: Everyone involved in the dairy community has the responsibility to aid in the improvement of both the quality of the employee's work environment as well as the welfare of the dairy animal. Dairy CARE 365 addresses both of these needs, and we have had many testimonials regarding increased worker retention, decreased worker and cattle injuries. Certainly the penetration of this program, if nothing more, illustrates the thirst that the dairy community has for employee education. Further research is needed in areas of improving delivery of adult education materials, retention of knowledge, and survey methodology to assess their impact.

Animal Welfare

P02-002-035

An Analysis Of The Drivers Of Nsaid Use Amongst Dairy Farmers And Dairy Veterinarians

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Objectives: Non steroidal anti inflammatory drugs (NSAIDs) have well recognised positive effects on animal welfare through the reduction of pain and inflammation, yet the level of NSAID use across farmers is highly variable. Up to a 15 fold difference in the level of NSAID use between low and high users has been documented. The greatest use of NSAIDs is probably in the dairy industry, yet even here, the drivers for dairy farmers' use of NSAIDs in farm animals are not well understood. This study was designed to explore and quantify these drivers.

Materials and Methods: NSAID sales data was collected from the clinic sales database. The volume of NSAID sales was converted into animal daily doses (ADD) to account for the differences in the duration of effect between short acting and long acting NSAIDs, and was standardised by dividing the ADD value by herd size. Farmers were then ranked into three groups based on the level of NSAID use. Furthermore, clients were ranked according to the level of engagement they have with their veterinarian (A, B and C)

An online survey (SurveyMonkey) was set up to investigate potential factors that may influence farmers' use of NSAIDs in animals. The questionnaire was structured to understand a farmer's own perception of pain, their animals', the farmer's understanding of the benefits of NSAIDs, and their demographic information on the likelihood of NSAID use. In addition, a survey for veterinarians was created using an identical format and questions. Furthermore, the veterinarian survey contained several veterinary targeted questions.

Proportions were calculated from categorical data and qualitative data were converted into a numerical scale for the calculation of a mean response score. R was used for data manipulation and analysis.

Results: A total of 49 farmers and 32 veterinarians responded to the survey. High and medium NSAID users tended to have less dairying experience (<11 years) compared with low NSAID users. High NSAID users were more likely to be an "A" client and are engaged with their veterinarian, whereas low NSAID users were more likely to be a "C" client and less engaging with their veterinarian.

Animal welfare was the most important factor that influenced the use of NSAID in animals. The importance of animal welfare increased with the level of NSAID usage.

Veterinary advice and return on investment were also important overall factors, however no trends were observed between the NSAID user groups.

The cost of the product, with holding periods, and non veterinary advice were more important for low NSAID users. A decreasing trend in the importance of these three factors was observed from low users to high users.

Low NSAID users tended to have greater experience in the dairy industry.

In addition to animal welfare, published literature and product potency were important factors for veterinarians. Administration route and free give-aways were not important factors for NSAID use.

There was an increased trend in the level of perceived animal pain from low users to high users and veterinarians. Veterinarians perceived a lower level of pain during caesarean section, gut surgeries and sick cows compared with farmers. In contrast, the level of perceived personal pain (human experience of painful conditions) did not vary between low and high users.

Conclusions: These data are of significant value in identifying the motivations of high and low NSAID users, and understanding some of the barriers to better amelioration of pain and inflammation in dairy cattle. For example, as NSAIDs were only introduced in the last decade or so, older and more experienced farmers may not be as familiar with their use compared with younger clients with more recent education within the dairy industry.

Engagement with veterinarians was highly correlated with use, suggesting that education of lower users needs to take account of these factors.

Animal Welfare

P02-002-036

Effect Of Different Combinations Of Local Anaesthetic, Sedative And Non-Steroidal Anti-Inflammatories On Growth Rate Of Dairy Calves After Disbudding

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Objectives: The aim of this study was to assess the effect of different forms of analgesia at disbudding on subsequent weight gain of dairy calves. Two different non-steroidal anti-inflammatories with different retail cost profiles were used and the study was designed to provide data which could demonstrate to farmers that improving analgesia is associated with better profitability as well as better calf welfare. It was hoped this would lead to an increase in the number of calves receiving analgesia at disbudding.

Materials and Methods: All calves were disbudded by trained personnel, using six disbudding protocols on 271, 3- to 6-week-old Friesian-Jersey calves. Three people disbudded 136 calves without sedation or local analgesia (non-veterinary method), but 31 received 20 mg meloxicam S/C and 75 received 150 mg ketoprofen I/M. Three different people disbudded 135 calves with sedation and local analgesia (veterinary method), and 30 also received 20 mg meloxicam S/C and 75 received 150mg ketoprofen I/M. Calves were weighed 14 and 3 days before disbudding, 15 and 30 days later. Daily weight gain was analysed using mixed models and ANOVA.

Results: From disbudding to Day 15 calves disbudded with the non-veterinary method receiving NSAID grew faster (0.66 kg/day) than calves without NSAID (0.53 kg/day; $p=0.031$), and there was no significant difference in the effect of either NSAID. An interaction between method and NSAID treatment ($p=0.001$) meant that NSAID treatment did not increase growth rate in veterinary-disbudded calves (0.64 vs. 0.65 kg/day, respectively; $p=1.000$).

From Days 16–30 there was no significant effect of NSAID on growth rate, but veterinary-disbudded calves grew faster (0.74kg/day) than calves disbudded with the non-veterinary method (0.66 kg/day; $p=0.018$). This meant that, if NSAID was not used, over the first 30 days calves disbudded with the veterinary method grew faster than calves disbudded with the non-veterinary method ($p=0.039$). However, if NSAID was used at disbudding there was no difference in growth rate between veterinary and non-veterinary-disbudded calves ($p=0.831$).

Conclusions: Three to 6-week-old dairy calves disbudded with the non-veterinary method with no analgesia grew significantly more slowly over the next 15 days than calves disbudded with the non-veterinary method given NSAID and more slowly over the next 30 days than calves disbudded by the veterinary method given xylazine and lignocaine. However addition of NSAID to the latter protocol had no effect on growth rate. This study adds to the evidence that analgesia during disbudding is beneficial for calf productivity as well as calf welfare.

Comments: In New Zealand, disbudding is usually carried out when the horn buds are 5–10 mm long and can be removed with a heated disbudding iron alone. The procedure may be performed without analgesia even though there is clear evidence that the procedure is painful. The cost of analgesics and the perception that there is no economic benefit to their use is often cited as one of the major influencing factors that motivate producers to not use analgesics in painful procedures.

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Animal Welfare

P02-002-037

Welfare of dairy cattle in the smallholder (zero-grazing) production systems in Nairobi and its environs

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Objectives: About 70% of dairy production in Kenya is from the smallholder production systems. These production systems are negatively impacted by a number of factors including poor nutrition, substandard husbandry and management practices, lack of appropriate farm inputs, diseases and low incomes. These factors influence the welfare of dairy cattle, hence their importance for its evaluation. This study was designed to determine the welfare of dairy cattle, risk factors and their indicators in the smallholder production units in Nairobi and its environs. Furthermore the farmers' and stockmen's perspectives of animal welfare were assessed.

Materials and Methods: These objectives were achieved through a cross-sectional study carried out in 80 smallholder dairy units purposively selected in Nairobi and its environs, in which 306 dairy cows were examined. The welfare of cattle in these dairy units was evaluated through several methods which included: visual observations

for animal- and farm-level factors that indicate poor welfare of cattle; taking measurements of dairy housing unit dimensions such as cubicle, walk-alley, kerb and feeding bunk; and using a structured questionnaire to interview farmers and stockmen on nutritional regimes and other management practices such as removal of slurry, milking techniques, record keeping and disease control. These factors were recorded and later analyzed. Analyses included descriptive statistics, and simple associations using chi-square at $p<0.05$ significance level.

Results: Over 80% of these smallholder units had factors that contributed to poor welfare of dairy cattle. These factors included under-size cubicles, small walk-alleys, too high feeding bunks with traumatic edges, too low positioning of neck rails at the feed bunks, sharp objects and edges within the housing units and dilapidated housing structures. The main evidence of poor welfare was injuries on the animals. The body condition score (BCS) of the cows was the main indicator of welfare relating to feeding. Presence of injuries or scars on the skin at various parts of the body was considered a positive indicator of poor welfare either associated with housing structures, management practices or animal interactions. Other causes of poor welfare of the cows were hind-limb tying during milking, teat pulling during hand-milking, more than 24-hour delay before sick cows were treated, and mixing of cattle of different age-groups in the same compartment. Cow-human interaction was poor as evidenced by fearful response and long avoidance distance.

Conclusions: This study concludes that poor welfare of dairy cattle exists in all the smallholder units evaluated, which is mainly caused by improper housing and management. Training of farmers and stockmen on animal welfare issues would therefore be a prerequisite to the improvement of dairy cattle welfare. Research on the physiological response to poor welfare of dairy cows in the smallholder units needs to be carried out to enhance the understanding of the impact of these risk factors on smallholder dairy animals.

Animal Welfare

P02-002-038

Fitness for transport - how do we assess it?

An observational study of culled dairy cows

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Objectives: In order to prevent pain and suffering during and after transport according to EU legislation, an animal must be fit for transport. Fitness for transport is, however, difficult to assess as not only the current condition of the animal must be assessed but also the potential effect of the transport on the animal's welfare. Both farmers, drivers and veterinarians find this challenging. The legislation offers only very broad and unspecific guidelines and limited research has dealt with the concept of fitness for transport. This study investigates if and how fitness for transport of dairy cows decided to be culled can be assessed.

Materials and Methods: This is an observational study including approximately 400 culled dairy cows transported to slaughter. The cows have been selected by farmers at 23 Danish dairy farms as a part of their normal culling routine and are transported by their regular trucking company to the slaughterhouse. A veterinarian examines the cows before and after transport. Examinations include behavioural and clinical observations e.g. grinding of teeth and locomotion score. Information

regarding the transport e.g. distance and duration is also recorded along with production data such as parity and gestational age. Data analysis will include a comparison of the examinations made before and after transport and potential risk factors for cows unfit for transport will be identified.

Results: The study is expected to generate knowledge about the general condition of culled dairy cows in Denmark and to identify behavioural and clinical manifestations prior to transport, to be used for fitness assessment. All transports are short and national with an average distance around 150 km and an average duration around 200 min. Preliminary results show that more than 1 out of 4 cows receive a higher locomotion score after transport compared to before transport. This suggests that culled dairy cows have an increased risk of becoming lame or more lame by being transported, even if the transports are relatively short.

Conclusions: Depending on the results it may be possible to develop a practical and reliable tool for assessment of fitness for transport. Such a tool could be of use to farmers, drivers and veterinarians helping them to assess which cows are fit for transport and which cows are not, and hence lead to better welfare for cows transported to slaughter.

Animal Welfare

P02-002-040

The Effect Of Topical Anaesthetic On The Sensitivity Of Scoop Dehorning Wounds In Beef Calves

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Objectives: The objective of this study was to assess efficacy of two newly formulated topical anaesthetics, specifically designed for application to dehorning wounds where excessive haemorrhage is often present, were compared in part 1 of a 2 stage study.

Materials and Methods: In part 1 of the study twenty-one Holstein dairy calves (16 – 20 weeks old) were randomly allocated to (1) scoop dehorning with a post-operative application of a topical anaesthetic powder and (2) scoop dehorning with a post-operative application of an evaporative alcohol / water solvent system spray. Wound anaesthesia was assessed by scoring the behavioural response of calves to wound touch stimulation on a numerical rating scale of 0 to 3. Wound anaesthesia was assessed immediately prior to treatment and 1, 90 and 180 min post treatment.

Based on the results obtained from part 1, a second study was conducted (part 2) as follows; the evaporative spray was compared to a commercially available topical anaesthetic, registered for use on mulesed sheep. Thirty-six Hereford beef calves (16 – 20 weeks old) were randomly allocated to (1) sham dehorning (CON, n = 7); (2) scoop dehorning with a pre-operative nerve block of lignocaine (DLA, n = 7); (3) scoop dehorning with a post-operative application of commercial topical anaesthetic (DTA, n = 7); and (4) scoop dehorning with a post-operative application of novel topical anaesthetic (DNNTA, n = 7). Wound anaesthesia was assessed as previously described immediately prior to treatment and 1, 2, 4 and 6 hours post treatment.

Results: The initial study (part 1) showed the calves treated with the evaporative spray responded less severely to touch stimulation at 90 and 180 min post treatment (P < 0.001). Therefore, in part 2 of the study, the evaporative spray was compared to a commercially available topical anaesthetic. There was a significant interaction between time and

treatment (P < 0.001) with CON calves displaying no to mild responses at all time points and all dehorned groups displaying increasing responses, yet not differing from one another.

Conclusions: The effect of topical anaesthetic is comparable to that of a nerve block of lignocaine and may be a more practical option for on farm use. In extensively managed production systems scoop dehorning of beef cattle reduces the risk of injury to stock workers and other cattle and decreases bruise trim at slaughter. As a result of distances, animal numbers and expenses, impracticality of currently available pain relief prevent widespread uptake for routine husbandry operations. Effective anaesthetic that can be applied topically would offer a more feasible option for producers to address the post-operative pain of dehorning.

Animal Welfare

P02-002-041

Reactivity variation over time of beef cows undergoing weaning

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Objectives: This study aimed to verify the continued responsiveness of beef cows, which were subjected to the stress of weaning, against a management practices commonly used in the farms, how to be restrained in a squeeze chute.

Materials and Methods: The experiment was conducted in Dom Pedrito, RS, Brazil, using 49 cows Braford, Brangus, Hereford and Angus breeds, raised in extensive system, exclusively on natural pasture. The animals were evaluated in three stages, with an interval of two months between each evaluation; by using the composite score (EC). The composite score was calculated based on the evaluation of animal mobility (1 = calm, 2 = active, 3 = restless, 4 = disturbed and 5 = very upset) and audibility and intensity of breath, vocalization, kicks and tension of animals during their stay in balance. Animal composite score ≤2 were considered non-reactive and ≥3 were considered reactive. The data were submitted to ANOVA in SPSS18® Program.

Results: The composite score of reactivity of the cows showed a significant variation (P < 0.01), according to the time of the inspection, with the frequency of reactivity classification measured by the composite score in the three periods distributed as follows: 79.59% of cows classified as non-reactive and 20.41% classified as reactive in the first assessment, 97.96% classified as non-reactive and 2.04% as reactive in the second evaluation and 95.92% as non-reactive and 4.08 as reactive in the third assessment.

Conclusions: We can conclude that the reactivity of beef cows submitted to weaning has a tendency to decrease as time passes after it occurs. More work is needed to verify the repetition of results in longer periods.

Animal Welfare

P02-002-042

Comparing the effects of intranasal and intramuscular administration of xylazine on the pain response of calves

Sabrina Lomax¹, Dominique McCarthy¹, Peter White¹¹Faculty of Veterinary Science, The University of Sydney, Sydney, Australia**Objectives:** This study aimed to:

1. compare the effects of intramuscular and intranasal administration of the alpha-agonist xylazine on time to and depth of sedation in holstein dairy calves
2. assess the analgesic effects of intramuscular and intranasal administration of xylazine on the pain response of dairy calves to caudatey disbudding

Materials and Methods: 16 Holstein heifer calves (62 – 100 kg) were randomly allocated to (1) caudatey disbudding (D, n = 4); (2) intramuscular administration of xylazine (0.1 mg/kg) followed by caudatey disbudding (IMX, n = 6); and (3) intranasal administration of xylazine (0.3 mg/kg) followed by caudatey disbudding (INX, n = 6). Clinical indications of onset and duration of sedation were observed. These included muscle relaxation, ptialism, lowering of the head and eyelids, recumbency, reduced respiration and heart rate and delayed or absent alertness. Calf response to disbudding was scored using a scale of 0 – 3 (0 = no reaction; 1 = minor response, slight eye widening, ear flick; 2 = moderate response, eye widening, ear flicks, head movement, vocalization; and 3 = severe response, vocalization, full head movement, escape attempt). A latency to move test was conducted 20 min and 45 min post treatment to test calf response to human approach. The observer took 0.5m steps towards the resting calf, and the distance (m) at which the calf moved to avoid the human was recorded. It was hypothesized that increased sedation would equate to a closer approach distance.

Results: Onset of sedation was recorded at 5 min for both IMX and INX calves. IMX and INX calves displayed all clinical indications of sedation. There were no observed differences between IMX and INX calves in terms of the degree and duration of sedation. There was a significant effect of treatment on the behavioural response of calves to disbudding ($P = 0.02$). D calves had significantly greater response scores than IMX and INX calves. There was a significant effect of treatment on latency to move ($P = 0.01$). D calves were more sensitive to human approach, as indicated by the greatest distance (3.7m). INX had the slowest response of 1.4m, significantly shorter than IMX (2.2m).

Conclusions: These results indicate that intranasal delivery of xylazine at a dose of 0.3 mg/kg resulted in fast and effective sedation and analgesia of calves as compared to intramuscular administration. Xylazine administered in both forms resulted in significant analgesia for calves undergoing caudatey disbudding. This presents a potential method for on-farm analgesia to manage perioperative pain in livestock.

Animal Welfare

P02-002-043

Impact of oral meloxicam on circulating physiological biomarkers of stress and inflammation in beef steers after long distance transportation

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Objectives: Transportation stress can result in significant economic losses to producers due to decreased animal productivity and increased medication costs associated with sickness such as bovine respiratory disease (BRD). Meloxicam (MEL) provides pain relief and anti-inflammatory effects in cattle for several days after a single oral treatment. Our hypothesis was that MEL administration before shipping would reduce the impact of long distance transportation on circulating physiological biomarkers of stress and inflammation in beef steers.

Materials and Methods: Ninety-seven beef steers were blood sampled for baseline biomarker determination and then randomly assigned to receive either 1 mg/kg MEL (n = 49) or a placebo (CONT) (n = 48) *per os* prior to a 1,316 km transportation event lasting approximately 16 h. Calves were then blood sampled on arrival and 5 d later. Changes in the hemogram, circulating plasma proteins, total carbon dioxide (TCO₂), fibrinogen, substance P (SP), cortisol, haptoglobin (Hp)-matrix metalloproteinase-9 (MMP-9) complexes and tumor necrosis factor alpha (TNF α) between treatments over time were compared using a Mixed Effects Model with statistical significance designated as $P < 0.05$. ANCOVA was conducted to assess the relationship between circulating MEL concentrations and biomarker changes over time.

Results: An increase in neutrophil, platelet, monocyte, white blood cell and red blood cell counts occurred after transportation ($P < 0.0001$) and a decrease in lymphocyte count was observed ($P < 0.0001$). MEL treatment reduced the stress-induced neutrophilia ($P = 0.0072$) and circulating monocyte count ($P = 0.013$) on arrival. Mean corpuscle hemoglobin ($P = 0.05$), mean corpuscle volume ($P = 0.05$) and lymphocyte count ($P = 0.05$) was also greater in the CONT calves compared with MEL calves after transportation. Furthermore, HP-MMP-9 complexes, TCO₂, TNF α , plasma proteins and SP increased and cortisol decreased after shipping ($P < 0.01$). MEL treatment tended to reduce serum cortisol concentrations ($P = 0.08$) and there was evidence of a time-by-treatment interaction ($P = 0.04$). An inverse relationship between plasma MEL concentrations and circulation cortisol concentrations ($P = 0.002$), neutrophil ($P = 0.04$) and basophil counts ($P = 0.03$) was also observed.

Conclusions: The results suggest that meloxicam administration may reduce the impact of long-distance transportation on circulating physiological biomarkers of stress and inflammation in beef calves on arrival to the feedyard.

Animal Welfare

P02-002-044

Comparative time, behavior and economic differences associated with calves receiving a pour-on vs. an intravenous product

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Objectives: Intravenous (IV) route of drug administration requires proper animal restraint, familiarity with bovine anatomy, and potentially increases stress, time and costs compared to pour-on administration.

The objectives of this study were: 1) Determine differences in time required to administer a pour-on saline solution compared to inject IV; 2) Measure behaviors during and immediately post saline administration and evaluate for potential differences; and 3) Determine economic costs associated with both a pour-on saline and an injectable saline solution based on the time and supplies necessary for administration.

Materials and Methods: One hundred beef crossbred calves were selected and randomized to treatment ((IV) or Pour-On (POUR)) based on chute order prior to the study. Each calf was moved into a processing chute and restrained using a head gate. Calves randomized to POUR received 18 ml of sterile saline on their back to simulate the administration of a pour-on product. Cattle assigned to IV were additionally restrained using a halter to fasten their head to the chute and allow access to the jugular vein prior to saline IV injections. The time calves were restrained in the head gate was recorded and 3 scoring systems (vocalization score, chute score, and exit score) were used to capture potential changes in behavior/stress associated with treatment. Costs associated with labor and materials for each treatment were estimated.

Results: Results showed a reduction in chute time per animal of 36.45 seconds comparing IV injection (53.61 s) vs. pour-on (17.16 s). Estimated labor cost per animal were \$0.54 USD less for POUR vs. IV calves. Cost of material needed for IV administration (syringe and needle) was estimated at \$0.70 per animal. The total cost savings achieved by pour-on administration compared to an IV injections was estimated at \$1.24 (\$0.54 [labor] + \$0.70 [material] = \$1.24 [labor + material]). Behavior analysis showed calves receiving a pour-on administration had a higher ($P < 0.05$) probability of not vocalizing while in the chute and having a normal chute score.

Conclusions: The pour-on administration provided a total cost savings of \$1.24 (\$0.54 + \$0.70) per animal compared to an intravenous injection. Animal vocalization and excessive movement during processing at the chute has been associated with calves experiencing stressful conditions. Data from the present study indicate that calves receiving a pour-on administration were less stressed while in the chute compared to those receiving intravenously administered saline.

Animal Welfare

P02-002-045

Benchmarking Locomotion Score in 114 Dairy Farms in Northwestern of Spain

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Objectives: To describe locomotion scores and lameness prevalence as well as showing opportunities for improvement on locomotion scores through benchmarking in 114 dairy farms in the Northwest Spain (Galicia's region).

Materials and Methods: Farms (n=114) were sampled during 2015. All free-stall barns with cubicles randomly selected by local veterinarians. Herd size ranged from 20 to 244 cows however, the median across farms was 43 cows (Holstein).

Measurements were collected only once on every farm around the time of the first milking (7 to 9 am).

Cows were scored in the feeding alley for locomotion between 1 (sound) and 5 (severely lame) according to guidelines by Sprecher et al. (1997) assessment. Lameness cows were under locomotion score 3, 4 and 5.

Results were described through percentage of each score by farm, overall media, standard deviation (SD), quartiles: first (Q_1 : 25% data), second (Q_2 : 50% data, median), third (Q_3 : 75% data); and, variation across farms was measured by the Interquartile Range (IQR: $Q_3 - Q_1$; data dispersion).

Results: Percentage of each locomotion score among the 114 sampled farms is reported by Q1 - Q2 - Q3, IQR and media (minimum - maximum), as follow:

% Score 1: 47 - 56 - 65, 18 and 65 (23 - 100).

% Score 2: 11 - 15 - 24, 13 and 24 (0 - 57).

% Score >2: 2 - 5 - 8, 6 and 11 (0 - 60).

The average for each score is not far from the recommended ones (70% score 1, 20% score 2 and < 10% score 3 - University of Wisconsin-Madison). However, the variation across farms was high.

Regarding the data published in other areas of the world and independent of the scoring system, the average prevalence of lame cows is relatively small (Europe or USA range from 14% to 31% [(Wells et al., 1993; Whay et al., 2003; Sarova et al., 2011)]. However, due to the high variation observed across our farms, the average itself does not show the real situation.

Conclusions: Even the lameness prevalence of Northwest Spanish farms was similar to other regions as well as close to the recommendations, there was a high variation across farms. Therefore, the average value may not be a good measurement to evaluate farms performance among different studies, while other descriptive statistics suggested in this study, as Quartiles or IQR, may allow opportunities to improve lameness prevalence within and across farms.

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Animal Welfare

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White Blood Cell Count after Disbudding in Calves Treated with Photodynamic Therapy

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Objectives: Photodynamic therapy (PDT) is a procedure that has been investigated for the treatment of microbial diseases, with no adverse effects. The antimicrobial properties from PDT combine a harmless photoactivatable dye with visible light and molecular oxygen. Its mechanism of action is based on the excitation of photosensitive substances by light at proper wavelengths, resulting in the formation of reactive oxygen species which will determine selective death of microorganisms. The general purpose of our research was to investigate the effect of PDT on calve's white blood cell count (WBCC) after disbudding, improving animal welfare.

Materials and Methods: Twelve male Holstein calves, four weeks old, disbudded by hot iron were used in the study. Local anesthesia was performed with lidocaine hydrochloride 2%. The cornual branch of the zygomaticotemporal nerve was blocked associated with infiltrative anesthesia around the cornual button. After hair clipping and antiseptics with 2% chlorhexidine, bilateral cauterization of germinal buds was made. For pain relief after disbudding, we administered metamizol sodic (25mg/kg, SID) during three days.

The animals were divided into three different treatment groups:

- 1) Control (ointment with zinc oxide) - 4 calves;
- 2) PDT: visible light - Red LED ($\lambda = 660\text{nm}$, 350mW, 4J) associated with a photosensitizer -methylene blue (0.01%) - 4 calves;
- 3) Visible light - Red LED ($\lambda = 660\text{nm}$, 350mW, 4J) - 4 calves.

Only one treatment with zinc oxide ointment, PDT and red LED was made, according to the described groups, immediately after hot iron disbudding.

Blood samples were collected for WBCC evaluation in seven different times: 1h before disbudding; 2h and 4h after disbudding; 3, 7, 14 and 21 days after disbudding.

Statistical analysis was made with Tukey's test ($p < 0,05$) using Minitab Statistical Software.

Results: According to our results, we haven't found statistical differences in the total leukocyte count among the different groups within the same time, as well as in the total number of neutrophils, eosinophils, basophils, lymphocytes and monocytes in any of the studied periods when different treatments were compared.

When the comparisons were made among the different times but within the same group, there was no difference as well.

Local burn injuries could lead to systemic alterations in WBCC if secondary infections and/or severe inflammation were present, but, in our study, there were no animals with systemic responses to them. Therefore, no alterations in WBCC were found regardless to the studied group.

Conclusions: We concluded that there was no difference in the WBCC in hot-iron disbudded calves, regardless the treatment group. Therefore, the WBCC did not present itself as an accurate indicator for acute inflammation in disbudded calves when local burn injuries remain without complications. Further studies are needed to assess the advantages of PDT as a practical and effective treatment after hot-iron disbudding in calves and to elucidate the potential role of PDT as wound healing promoter, improving the animal welfare after such procedure.

Animal Welfare

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Prevalence and Risk Factors for Hock Lesions in Swedish Dairy Cows

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Objectives: Hock lesions (HL) indicate poor cow environment and are often used in animal welfare assessment protocols. The lesions cause pain and discomfort, are associated with lameness, increased milk somatic cell counts (SCC) and culling rates, and are common in several countries. Few studies have, however, been performed in Sweden. Risk factors have been identified, but the etiology is still not fully understood. The objectives of the study were to investigate the prevalence of HL in Swedish dairy herds, to identify herd- and cow-level risk factors associated with HL and to analyze associations between HL and mastitis, claw disorders and culling.

Materials and Methods: A random sample of 100 Swedish dairy herds were visited once during winter/early spring 2014 or 2015. Included herds had free-stalls, 50-210 cows, milking parlor and were affiliated with the Swedish official milk recording scheme. Every second to third cow was examined during milking and registered as having no HL (no visual skin damage or hair abrasion), mild HL (hair loss) or severe HL (skin wound or evident swelling) on the outside of the hock visible when standing in the milking parlor. Examined cows were also assigned a hygiene score based on hind limb and udder cleanliness. Additional information on individual cows, herd management and cow environment factors was obtained both on farm via registrations and a pre-formed questionnaire, and from the official milk recording scheme. Investigated cow level risk factors included age, breed, days in milk, milk yield, milk urea levels, milk SCC, hygiene score and claw disorders. Veterinary-treated cases of clinical mastitis 30 days before and after the visit, and culling up to three months after the visit were registered to investigate their possible association to HL. Herd level risk factors investigated were herd size, production system (organic or non-organic), stocking density, cubicle size, bedding material, barn cleaning routines, claw trimming routines, average milk production level, breed, bulk tank milk SCC and urea levels, feeding routines, length of pasture period and registered veterinary treated diseases (with special regard to mastitis, claw disorders and feed-related disorders).

Results: The total cow prevalence of HL was 74% (2582 of 3775 cows), and the majority (91%) of the lesions were mild while 9% of the HL were severe. The within-herd prevalence of HL ranged between 24-100% (average 73%), while the within-herd prevalence of severe lesions ranged between 0-30% (average 6%). Several herds had a high (50-100%) proportion of mild lesions but no, or very few, cases of severe lesions, while other herds had high proportion of both severe and mild lesions. The combination of a high proportion of severe HL (>10%) and a low proportion (<50%) of mild lesions was unusual but seen in a few herds. Potential associations between HL and the risk factors are analyzed using univariable and multivariable regression models and these results will be presented at the congress.

Conclusions: The results indicate that the prevalence of HL in Swedish dairy herds is similar to other countries with equal production systems. Thus, HL is an important health problem also in Sweden. The large within-herd variation in prevalence and proportions of mild and severe HL indicate that herd factors may be important for the occurrence and severity of the lesions. If identified, these factors could be an important aid

in reducing herd prevalence of HL. Longitudinal and microbial studies of HL are of interest to increase understanding on why and how the lesions develop, persist and heal, and will also be performed within the project.

Animal Welfare

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Developing a farmers evaluation form to create awareness on the behaviour and pain management of Belgian Blue cows after a caesarean section

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Objectives: The use of non-steroidal anti-inflammatory drugs (NSAID) during a caesarean section is not a common practice among beef farmers with Belgian Blue cows. The objective of this field case study was to create and test an evaluation form for beef farmers on the behaviour and pain management of Belgian Blue cows after caesarean section using a NSAID. With this form awareness can be created on behaviour related to pain after a caesarean section in beef cows. This study was initiated and executed by a veterinary practice in the Netherlands.

Materials and Methods: A large veterinary practice in the south of the Netherlands enrolled their clients randomly in this field experience. The cows were operated using a standard operating procedure performed by all veterinarians in this practice. A NSAID (meloxicam 20mg/ml, 2.5ml/100kg subcutaneously) was administered by the veterinarian directly after the operation. To evaluate the effect of the NSAID on the behaviour a form was created. This farmer's evaluation form had to be practical and easy to use for a good response rate and reliability. Important parameters for a farmer are the lying or standing time, lying-standing bouts, feed intake, the suckling activity of the calf (in case there is no separation), the colostrum and milk production and the body temperature (°C) of the cow. In this field case study the following parameters had to be observed by the farmer: lying time, lying-standing bouts, feed intake of the cow and suckling time of the calf. The scores were divided in high, average and low. A table was present on the form with the four parameters on the y-axis and the three scores on the x-axis, by doing this the farmer could easily put a cross-mark in the cell of his choice. The observation period was day 1 and day 2 after the surgery. There was one open question on the form which stated "what is your general impression of the cow in the first two days after the caesarean with the NSAID treatment". Hereby the farmer could share his experience of the treatment. The farmers in this trial did not use a NSAID during a caesarean surgery previously.

Results: After the trial period (August 2015 until October 2015) eighteen caesarean sections were evaluated and these operations were done by 4 different veterinarians on 5 different farms. The scores high, average and low of the lying time on day 1 and day 2 combined were respectively 2, 18 and 16. The scores high, average and low of the lying-standing bouts on day 1 and day 2 combined were respectively 0, 23 and 13. The scores high, average and low of the feed intake on day 1 and day 2 combined were respectively 22, 12 and 2. The scores high, average and low of the suckling time of the calf on day 1 and day 2 combined were 17, 9 and 1. The total percentage of the scores on this evaluation form was 29.9% high, 45.3% average and 24.8% low. The average temperature of the cows on day 1 was 39.0 °C with a standard deviation of 0.5. The average temperature of the cows on day 2 was 38.8 °C with a standard deviation of 0.4. The open question on the form was used by every farmer. The general opinion was very positive; a quick recovery of the

cow with a good feed intake and suckling activity of the calf was noticed.

Conclusions: The response rate of this evaluation form was high; all the farmers who were asked to fill in this form returned it with every questions answered. A lot of the parameters were scored with average, this could be a sign of low interest of the farmer and possible poor reliability. The open question was answered on every form, so the farmers were motivated to share their positive experiences. Beef farmers were more aware of the behaviour of their cows after a caesarean section and the effects of pain management on this behaviour. A practical and easy to use evaluation form can serve as an excellent tool to achieve this purpose.

Animal Welfare

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A Pilot Study to Investigate the Feasibility of Behavioural Monitoring of Periparturient Cows and Calves on a Commercial Dairy Farm in Scotland.

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Objectives: Dystocia is a painful event for both the cow and the calf; however, there is a paucity of research in this area, particularly regarding the use of analgesia at the time of calving. The current project aims to investigate the use of non-steroidal anti-inflammatory drugs (NSAIDs) in both the cow and the calf at calving.

Results from a preliminary feasibility project investigating cow and calf behaviour post-partum undertaken in the summer of 2015 will be presented. The practicalities involved in performing such a project on a commercial dairy farm were assessed. A 700 cow herd in Scotland has been recruited for this study.

Materials and Methods: A camera recording system monitored cow and calf behaviours after calving. The degree of assistance required for calving was graded by the farm staff using an established dystocia scoring system (1=no assistance; 2=mild repositioning required but not excessive traction; 3=severe traction required; 4=veterinary assistance or caesarean section). Grade 4 calvings were excluded from the study. The observer was blinded to the degree of assistance required.

Calf study

Four cameras covering the calf pens monitored calf behaviour for the first 48hours after calving. Calves were moved out of the calving pen and into a calf pen as soon as practicably possible after calving. Any time spent with the dam was captured by cameras in the calving pen.

Video of the behaviour of 10 assisted calves and 10 non-assisted calves was analysed. An informal interval analysis performed at the beginning of the study indicated that observing calf behaviour for 5 minutes every other hour was an appropriate sampling interval.

Cow study

The same camera system (using 6 cameras) covered the calving and post-partum pens to monitor cow behaviour for 48hours post-partum.

Due to the longer period of time spent setting up the camera system in the calving and post-partum pens, cows were videoed over a shorter period than calves (2 weeks compared to 8 weeks). Cow behaviour was

analysed by a different observer using continuous observation for the first 2 hours post-partum. From 2 to 48 hours post-partum, behaviour was analysed using interval sampling; cow behaviour was observed for 10 minutes every hour.

Results:

Calf study

Behavioural analysis of calves indicated that the greatest proportion of time was spent exhibiting lying behaviours. This did not differ significantly between assisted calves and non-assisted calves ($89.78 \pm 3.98\%$ and $88.99 \pm 6.23\%$ (mean \pm sd) respectively; $p=0.98$).

Assisted calves did not participate in play behaviours as much as non-assisted calves. Non-assisted calves not only spent double the proportion of time showing play behaviour than assisted calves, ($0.81 \pm 1.45\%$ and $0.29 \pm 0.66\%$ respectively) with a degree approaching significance ($p=0.09$), they also exhibited more variation in play behaviours than assisted calves.

Cow study

Behavioural analysis of the cows indicated that cows with unassisted calving spent a greater proportion of time lying down on the day of calving than the day after calving. Cows that had an assisted calving showed more similar proportions of time spent lying down on both day 1 and day 2 post-partum. Due to the small sample size ($n=4$) no statistical conclusions can be made.

Conclusions: Although data were limited, the intended aim was achieved. It is possible to perform a study such as this on a commercial dairy farm. Further work will be undertaken during 2016 with an intervention study using NSAIDs at the time of calving in both cow and calf. Behavioural analysis using the model established during this study will be performed, as will assessment of physiological parameters and analysis of farm recorded data.

This study aims to address the lack of research in this area and in the future enable practitioners to advise their farm clients regarding analgesic use to improve the welfare and production of both cows and calves.

Animal Welfare

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Characterization of Cow Activity Behavior During the Transition Period

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Objectives: Previous research has shown cows experiencing postpartum disease events had lower feed intake in the weeks prior to calving compared to cows with no abnormal health events. More recent research has implicated alterations in cow feeding behaviors as an underlying cause of the feed intake decline and suggest prepartum feed intake could be used as an indicator of increased risk for postparturient disease. There are limited data documenting cow activity behavior during the transition period. The objective of this study was to characterize cow activity behavior during the dry period and early lactation of healthy animals.

Materials and Methods: A total of 178 dairy cows were continuously followed over a period of 6 months on a commercial 750-cow dairy from the time of entry into the dry cow group (average 60 d dry) through 3 weeks postpartum. Three-axis accelerometers (HOB0 logger, ONSET

Corp.) were placed on the hind leg of each cow, just above the fetlock joint, to record standing or lying position in 5 minute intervals. Cows were moved into the dry pen weekly and transferred to the close-up pen approximately 3 weeks prior to expected calving. Cows imminently calving were moved into the maternity pen. Heifers were housed in a single separate pen from cows and moved into the maternity at calving. After calving all cows were transferred to then the fresh cow pen within 6 hours. Dry, close-up and fresh pens were well-designed free stalls with deep sand bedding. Maternity pen was loose housing with straw bedding. All health observations, events and treatments were recorded using a computerized recordkeeping system (DairyComp 305®). Data from 84 cows with no abnormal health events were used for this analysis. Accelerometer data were transformed into behavioral activities of standing and lying times (hr/d), lying bouts per day (n), and average lying time per day (hr). Upper and lower 1% quantile data were censored to remove extreme values. Behavioral activities were analyzed by analysis of variance using repeated measures and regression modeling to account for parity (1st, $\geq 2^{\text{nd}}$), pen, time or day relative to calving, and calving month.

Results: Fresh cows spent less ($P<.0001$) time lying (10.3 ± 1.6 hr) compared to dry cows (12.8 ± 1.7 hr) and times did not vary ($P=.2$) by parity, though there was a tendency ($P=.08$) for cows to have longer times compared to heifers in dry and fresh periods. Number of lying bouts and average lying time were influenced by dry vs. fresh period ($P<.0001$, $.0001$), age ($P<.0001$, $.0001$), although there was a significant ($P<.0002$, $.01$) interaction between the two. Cows had less lying bouts and nearly twice the average lying times in all periods compared to heifers. Heifers increased their lying bouts ($n=8.7, 9.2, 10.2$) and decreased average lying time ($2.4, 2.0, 1.0$ hr) from dry to close-up to fresh periods, respectively. Day relative to calving influenced ($P<.0001$) lying time in the dry, close-up and fresh pens. In the dry pen, cows increased ($+0.03$ hr/d, $P<.0001$) lying time while heifer total lying time did not change. Cow lying time was increased by less lying bouts ($P<.0001$) with longer ($P<.0001$) average lying times. Both cows (-0.034 hr/d, $P=.007$) and heifers (-0.057 hr/d, $P=.0003$) decreased their lying time in the 3 wk prior to calving. Average lying time per bout was reduced in both heifers ($P<.0001$) and cows ($P=.009$). Fresh pen lying time increased ($+0.03$ hr/d, $P=.03$) in heifers and tended to increase in cows ($+0.02$ hr/d, $P=.09$). Cows first moved into close-up and fresh pens had decreased total lying time ($P=.01$) and average lying time per bout ($P=.0035$) in the 4 days upon pen entry. Greater negative effects occurred in the fresh pen.

Conclusions: These data show patterned changes in activity behaviors in healthy cows and heifers during transition, though measures are highly variable among individuals. Though cows and heifers have similar total lying times, different patterns of lying behavior occur in comingled cows and heifers during transition. Lying time was reduced for 4 days whenever a cow changed pens. These data provide some standards for evaluating transition cow behaviors in a commercial dairy with good cow comfort.

Animal Welfare

P02-002-051

The Healthy Calf – A Nationwide Survey Examining The Igg-Concentrations In Newborn Calves

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Objectives: Worldwide, the mortality of calves during the first weeks of life is still 10 to 15%. More than 80% of the calf losses can be traced back to farm-specific management problems. An improved colostrum management can help to reduce these calf losses. The objective of this study was to evaluate the IgG-Status of newborn calves in Germany.

Materials and Methods: Using a survey, data relating to the farms were collected such as the number of animals, type of housing, illnesses in the barn, especially diarrhea, and specific calf-related data such as breed, parity of the dam, calving difficulties, age of calf at blood withdrawal, time spent with the dam and the time after birth, quantity and method in which the colostrum was fed. Additionally, data regarding colostrum monitoring were recorded. The Immunglobulin G (IgG) content of altogether 1242 blood samples (taken between the 36th to the 96th hour of the calves' life) from 296 farms all over the country were taken from February to September 2015 and were analyzed using a Sandwich ELISA (Erhard et al., 1995). To trace back the health status of the examined calves within the first eight weeks of life the farmers were asked to complete another questionnaire concerning the occurrence of diarrhea and Pneumonia and the time at which the calves fell ill.

Results: The results show that nearly 23% of the calves were completely (failure of passive transfer, FPT, IgG-concentrations < 5 mg/ml serum, n= 284) and 36% partly (partial failure of passive transfer, pFPT, IgG-concentrations 5-9.9 mg/ml serum, n= 450) undersupplied with IgG. Only 41% of the tested calves showed sufficient IgG-concentrations (> 10 mg/ml, n= 508).

The number of lactations effected the serum IgG levels of the newborns ($p < 0.001$, Pearson correlation $R = 0.149$) slightly positive.

The method by which colostrum was fed did not significantly influence the calves' serum IgG concentrations. The Median IgG-levels ranged from 8.3 mg/ml (bottle), over 8.4 mg/ml (bucket) to 8.5 mg/ml in the drenched calves. The amount of colostrum fed during the first feeding had a significant influence ($p < 0.001$, Pearson correlation $R = 0.144$) on the serum IgG concentrations of the calves. There was no significant difference ($p = 0.182$) between the calves which became diarrhea and those without diarrhea in the first weeks of life. However, calves with diarrhea showed higher IgG-concentrations (Median with diarrhea: 10.05 mg/ml; without diarrhea: 8.05 mg/ml). The same trend could be seen with Pneumonia. Calves with Pneumonia had also higher IgG-levels (Median with Pneumonia: 11.10 mg/ml; without Pneumonia: 8.65 mg/ml, $p = 0.490$).

Conclusions: In conclusion there are still needs for clarification and action concerning the management of calf rearing in Germany and most likely in other countries as well.

This study was initiated and conducted in cooperation with MSD Animal Health, Germany.

Animal Welfare

P02-002-052

Novel methods to alleviate the peri-operative and post-operative pain of amputation dehorning in beef calves

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Objectives: The aim of the study was to assess the efficacy of a pre-operative buccal sedative / non-steroidal anti-inflammatory drug (NSAID) formulation, a pre-operative cryo-anaesthetic (CA) and a post-operative topical anaesthetic (TA), for pain alleviation in calves undergoing amputation dehorning. Efficacy was evaluated through assessment of behaviour, inflammatory indicators and wound healing.

Materials and Methods: The experimental protocol was conducted under institutional animal ethics committee approval. Seventy-five Hereford calves, 5 months of age, were randomly allocated to (1) sham dehorning / control (CON, n = 15), (2) amputation dehorning with a pre-operative local nerve block of lignocaine (currently perceived as industry best practice) (DL, n = 15), (3) amputation dehorning with pre-operative application of a cryoanaesthetic (DC, n = 15), (4) amputation dehorning with pre-operative administration of a buccal sedative / NSAID (DSN, n = 15), and (5) amputation dehorning with post-operative application of a TA (DTA, n = 15). Adverse calf behaviour during the dehorning procedure was scored on a numerical rating scale as a measure of peri-operative pain. Calf behaviour was also monitored for 6 hours post treatment. Events that were recorded include head shaking, ear flicking, tail flicking, head turning, leg to head pawing and head rubbing. States that were recorded include neck extension, scratching, normal lying, abnormal lying, normal standing, abnormal standing, normal walking and abnormal walking. The frequency of events and states were recorded and additionally the time length of states was recorded. Measurements for assessment of inflammation and wound healing of the horn area were taken immediately prior to treatment and 1, 3 and 7 days post treatment. Inflammation and wound healing were assessed via infrared thermography, measurement of wound contraction, plasma haptoglobin and observational scoring of the horn area on a numerical rating scale.

Results: DL and DC calves displayed less adverse behaviour during the dehorning procedure compared to D, DSN and DTA calves ($P < 0.05$). In the 6 hours following treatment, DSN and DTA calves displayed significantly less pain related behaviour than DL and DC calves ($P < 0.05$). Wound temperature and contraction varied across days ($P < 0.05$). There was no treatment effect on wound temperature or contraction. Healing scores significantly increased on day 7 ($P < 0.05$). There was no significant treatment effect on wound healing score. DSN calves had overall reduced concentrations of haptoglobin compared to all other dehorned calves ($P < 0.05$).

Conclusions: The nerve block of lignocaine and the CA reduced adverse behaviour during the dehorning procedure, suggesting that they both mitigate peri-operative pain. CA is quicker and easier to use than administering nerve blocks and therefore may be a more practical option for on farm use. Buccal sedative / NSAID and TA reduced acute pain related behaviour during the 6 hours following treatment. In addition, the buccal sedative / NSAID reduced haptoglobin concentrations, suggesting a reduction in inflammation and associated pain. Buccal and topical delivery of analgesia may provide farmers with feasible options of providing pain relief for dehorning.

Animal Welfare

P02-002-053

Efficacy of a topical anaesthetic (TA) and a buccal non-steroidal anti-inflammatory drug (NSAID) for pain relief of surgically castrated calves.

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Objectives: To investigate the effect of a topical anaesthetic (TA) and a buccal non-steroidal anti-inflammatory drug (NSAID), alone and in combination, on post-operative behaviour, scrotal temperature, scrotal size, plasma haptoglobin and wound healing of beef calves following surgical castration. This investigation aimed to assess the use of TA and buccal NSAID as effective and practical forms of pain relief for incorporation into routine farm management.

Materials and Methods: The experimental protocol was conducted under institutional animal ethics committee approval. Fifty Angus bull calves, 3 months of age, were randomly allocated to (1) sham castration / control (CON, n = 10), (2) surgical castration (C, n = 10), (3) surgical castration with pre-operative administration of a buccal NSAID (CN, n = 10), (4) surgical castration with post-operative application of a topical anaesthetic (CTA, n = 10) and (5) surgical castration with pre-operative administration of a buccal NSAID and post-operative application of a topical anaesthetic (CNTA, n = 10). Animal behaviour was monitored for 6 hours post treatment. Events that were recorded include tail flicking, head turning, foot stamping, kicking and easing quarters. States that were recorded include hind leg extension, scratching, normal lying, abnormal lying, normal standing, abnormal standing, normal walking and abnormal walking. The frequency of events and states were recorded and additionally the time length of states were recorded. Measurements for assessment of inflammation and wound healing of the scrotal area were taken immediately prior to treatment and 1, 2 and 7 days post treatment. Inflammation and wound healing were assessed via infrared thermography, measurement of scrotal size, plasma haptoglobin and observational scoring of the scrotal area on a numerical rating scale.

Results: In the 6 hours following treatment, CN, CTA and CNTA calves displayed significantly less pain related behaviour than C calves ($P < 0.05$). Both average and maximum scrotal temperature varied across days ($P < 0.05$). There was no treatment effect on scrotal temperature. Scrotal size of castrated calves increased in the following 2 days post treatment and then decreased on day 7 ($P < 0.05$). Healing scores significantly increased on day 7 ($P < 0.05$). Administration of the buccal NSAID and the TA had no effect on scrotal size or healing score. CN and CNTA calves had overall reduced concentrations of haptoglobin compared to C and CTA calves ($P < 0.05$).

Conclusions: Reduced acute pain related behaviour indicates that both buccal NSAID and TA ameliorate the post-operative pain of surgical castration in beef calves. Lowered haptoglobin concentrations of CN and CNTA calves suggest that buccal NSAID reduces the inflammatory response to surgical castration. Both buccal NSAID and TA are practical to use on farm and therefore may be readily adopted by producers as options for pain relief during routine surgical husbandry procedures such as castration.

Animal Welfare

P02-002-054

The effect of pain relief at cautery disbudding on stress, behaviour, and growth rate in dairy calves and farmer perception of these methods

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Objectives: 1) To assess the behavioural response of calves to cautery disbudding. 2) Assess the effectiveness of sedation, cornual nerve block and oral NSAID administration at mitigating pain, pain behaviour and stress during disbudding. 3) Assess farmer perception on calf welfare at disbudding and to assess willingness of farmers to adopt pain relief into disbudding protocols.

Materials and Methods: Healthy Holstein Friesian dairy calves (n=124) were selected from a dairy farm in the Finley region of Southern NSW. The calves were allocated to one of the following groups:

Control 1- Calves restrained in disbudding crush and 'sham' handled without disbudding being performed. Control 2: Calves sedated, cornual nerve block performed and oral NSAID administered and 'sham' handled without disbudding being performed. Treatment 1: Cautery disbudding without the provision of sedation or pain relief, Treatment 2: Cautery disbudding with the provision of pain relief (sedation, cornual nerve block and oral NSAID administration) with horn bud removal and Treatment 3: Same as treatment 2 pain relief without horn bud removal. Calves were weighed prior to the trial, 48 hours following the trial and weekly for the next 2 weeks post disbudding to assess immediate and long term changes in average daily gains. The behavioural reaction of calves to disbudding was assessed and ranked using an agitation scale (0-5). The behaviour of calves was observed for 6 hours following the procedure. Frequencies of behaviours indicative of pain such as; head shaking, ear flicking, head rubbing and foot stamping were recorded, as were behaviours considered normal in calves such as; ruminating, drinking milk and eating straw/grain. The time taken to complete each treatment was recorded to assess the time efficiency of each method. Following a demonstration of disbudding methods farmers voluntarily completed a questionnaire regarding calf welfare at disbudding and willingness to adopt pain relief protocols.

Results: There was a significant ($P \leq 0.05$) increase in average daily gain (Treatment 1= 601 g/d, Treatment 2= 666 g/d, Treatment 3 = 776.8 g/d) in dairy calves over the 2 week period post disbudding for Treatment 2 compared to Treatment 1. The provision of pain relief was also associated with a significant reduction in agitation score at the time of disbudding and calves exhibited fewer behaviours, in the 6 hours post treatment, associated with pain compared to the no pain relief group.

The results of this study show that there was significant effect on welfare and ADG in calves following disbudding procedure when sedation, a local anaesthetic and an oral NSAID was incorporated into the cautery disbudding protocol.

Based on the questionnaire survey from farmers (N= 28) who observed the procedure, it was shown that 71.4% of farmers thought that pain relief at disbudding was helpful and were willing to incorporate the protocol of pain relief on their farm. Furthermore, 50% of farmers identified it as an avenue to improve the public perception of dairy farming. They agree that such methods provide a higher standard of welfare, however do hold concerns regarding the cost of such protocols.

Conclusions: It can be concluded from this study that incorporating pain relief into common husbandry procedures such as disbudding is beneficial,

both in regards to animal welfare and production. This study has also identified that producers are willing to accept and adopt these protocols to improve the welfare of their animals and also to improve the public perception of the dairy farming industry. Future studies to identify the most effective disbudding technique and investigation into avenues to reduce the cost associated with incorporating pain relief protocols into common husbandry procedures is warranted.

Anti-Microbial Resistance

P03-003-001

Consequences of Virginiamycin Used as Growth Promoter on the Prevalence of Antibiotic Resistant *E. coli* in Feces of Feedlot Cattle.

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Objectives: This study aimed to investigate the prevalence of antibiotic resistant bacteria present in feces of feedlot cattle before and after the use of virginiamycin given as antibiotic growth promoter (AGP) and to compare treated animals with a control group.

Materials and Methods: Thirty-six crossbreed cattle (*Bos taurus taurus* x *Bos taurus indicus*) were randomly assigned to one of two groups (n=18 per group): animals treated with virginiamycin (ATB) and a control group (CON) receiving the same diet without the antibiotic. Fecal samples were collected at arrival and exit from a feedlot operation. Isolation of *Escherichia coli* was done by standard methods and 2 to 3 colonies from each plate were tested for antimicrobial resistance using the disk-diffusion method, as recommended by the Clinical and Laboratory Standards Institute guidelines. The following antimicrobial agents were selected: amoxicillin-clavulanic acid, ampicillin, aztreonam, ceftiofur, chloramphenicol, ciprofloxacin, enrofloxacin, gentamicin, nalidixic acid, streptomycin, tetracycline and trimethoprim-sulfamethoxazole. Fisher exact test was used to compare the prevalence of resistant isolates at the different sampling times and between groups (CON vs. ATB) within each moment.

Results: Two hundred six *E. coli* colonies were analyzed, of which 98 were from samples collected at arrival (CON=50 and ATB=48) and 108 collected at exit (CON=54 and ATB=54). Only 2% of isolates from arrival and 0.2% from exit showed resistance to one of the antimicrobial agent tested. At arrival, one isolate from ATB and two from CON were resistant to nalidixic acid; four from CON had intermediate resistance to amoxicillin-clavulanic acid; four from CON and one from ATB had intermediate resistance and two from CON and one from ATB were resistant to ampicillin; one from CON was resistant to enrofloxacin; six from CON had intermediate resistance to streptomycin; and two from CON and one from ATB were resistant to tetracycline. Four isolates, three collected at arrival (two from CON and one from ATB) and one at exit (CON), were resistant for both, ampicillin and tetracycline. All isolates were sensitive to aztreonam, ceftiofur, ciprofloxacin, chloramphenicol, gentamicin and trimethoprim-sulfamethoxazole.

At exit, only one isolate had intermediate resistance and one was resistant against ampicillin, and one against tetracycline (all isolated from the CON group). There was no significant difference in antimicrobial resistance between animals comparing arrival to exit, neither ATB and CON at exit (P>0.05 for all the comparisons).

Conclusions: The sensitivity to several antibiotics tested in the samples collected for this study is suggestive that at these conditions, the use of virginiamycin used as AGP may not be of public health concern for the development of multidrug resistant bacteria.

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Anti-Microbial Resistance

P03-003-002

Monitoring of antibiotic dispensing by veterinarians in farm animal practice in Austria

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Objectives: The Austrian Federal Ministry of Health, in common with other health authorities worldwide, is concerned about the use of antibiotics in both human and veterinary medicine. In order to be able to assess the current situation in Austria with regard to antibiotic use in food-producing animals, a new law was passed requiring veterinarians to report all antibiotics dispensed on farm. The University of Veterinary Medicine is also involved in a study as part of the ADDA (Advancement of Dairying in Austria) project, collecting data on antibiotic administration by veterinarians, with particular respect to the treatment of dairy cows with mastitis.

Materials and Methods: The Austrian “veterinary antibiotic volume flow” regulation (Veterinär-Antibiotika-Mengenströme-Verordnung) was enacted in 2014. Veterinarians treating food-producing animals are required to report all antibiotics dispensed to farmers for each calendar year. The first reports are due to cover the period 1st January 2015 - 31st December 2015. Veterinarians are required to transfer the data to the Austrian Agency for Health and Food Safety (AGES). In addition, pharmaceutical companies and wholesale pharmacies were required to provide details of antibiotics delivered to veterinarians from 2014 onwards. Furthermore, farmers can voluntarily provide details of antibiotic use and administration on farm.

Results: To date, all pharmaceutical companies/wholesale pharmacies operating in Austria have provided data on their supplies of antibiotics to veterinarians in farm practice. A total of 1733 veterinary practices are registered as having their own in-house pharmacy in Austria. Of these, 1626 ordered deliveries of antibiotics from wholesalers/pharmaceutical companies in 2014. The majority of antibiotics were supplied to a relatively small number of veterinary practices: 95% of the total volume of antibiotics ordered were delivered to just 328 practices (equivalent to 20% of the total number receiving antimicrobial products). The mean volume of critically important antimicrobials (fluoroquinolones, macrolides and 3rd-4th generation cephalosporins) made up 8% of the total volume ordered.

Cattle, sheep, goats, pigs, deer, poultry, llamas, ostriches, farmed fish, rabbits and horses are all classified as food-producing animals and veterinarians dispensing antibiotics for use in these species must report this. A number of producer organisations (such as the Poultry Association) also require their members to report antibiotic administration on farm, which goes beyond the requirements of the law.

With respect to the ADDA (antibiotic use in dairy cows) project, data collection began on 1st October 2015 and will continue until 30th September 2016. To date, 17 veterinary practices and approximately 280 dairy farms are included in this project. Data are expected to cover antibiotic administration in 7000 dairy cows (15,000 cattle in total).

Conclusions: Austrian laws regarding the dispensing of prescription-only medications by veterinarians are already much stricter than those in many other European countries. The Veterinary Medication Control law (Tierarzneimittelkontrollgesetz) precisely limits which medications can be left by veterinarians on farm. From 2016 onwards, it will be possible to analyse antimicrobial dispensing data by animal species and production method, which will provide additional insights into farm animal practice in Austria.

Anti-Microbial Resistance

P03-003-003

Multi-Drug Resistant Coagulase-Negative Staphylococci On A Single Farm In Australia

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Objectives: The objectives of this study were to test the antimicrobial susceptibility profiles of 37 isolates of coagulase-negative staphylococci (CoNS) from a single dairy farm in South Australia, Australia, battling a high rate of mastitis treatment failure. The testing was carried out with aims to detect the potential emerging resistance in CoNS in Australia, on a farm that heavily uses antimicrobial treatment for mastitis cases occurring in cows during lactation.

Materials and Methods: Isolates originated from 37 milk samples from cows aged 2-10 years. All samples originated from quarters that have been recently or inter currently treated for mastitis by the farm staff. The total number of submitted samples was 320 quarter-level samples from a total of 87 cows. This study concentrates on the results obtained from the 37 isolated samples of CoNS.

A pure culture of each isolate was frozen after culture from the original milk sample was stored at -80°C and used for MIC determination and confirmation of species identity by 16S rRNA sequence analysis.

Antimicrobial agents (ampicillin, cefotaxime, chloramphenicol, ciprofloxacin, gentamycin, neomycin, novobiocin, oxacillin, vancomycin, spectinomycin, streptomycin, tetracycline, and tylosin; Sigma, Australia) and antimicrobial disks (ampicillin, cefotaxime, ceftiofur, chloramphenicol, gentamycin, neomycin, novobiocin, and oxacillin; Oxoid, Australia) were obtained for determination of the minimum inhibitory concentration (MIC) of each isolate.

Minimum inhibitory concentrations (MIC) were determined using the broth microdilution technique.

From each isolate bacterial DNA was extracted by growth overnight in 5 mL Muller Hilton broth using a DNA extraction kit (Mericon DNA Bacteria Kit, Qiagen, Australia) according to the protocol recommended by the manufacturer. The extracted DNA was used for detailed identification and detection of resistance genes.

Results: A total of 37 isolates were confirmed as CoNS. The identification of these isolates found *S. xylosus* (n=9), *S. haemolyticus* (n=8), *S. succinus* (n=6), *S. fleurettii* and *S. sciuri* (n=4 of each), and *S. chromogenes*, *S. equorum*, and *S. simulans* (n=2 of each). The large proportion of the isolates obtained from these treated quarters was CoNS (28%). This should raise a question if they should be still assumed as minor or opportunistic pathogens, or it is time to discuss the need of speciation during the routine laboratory diagnostics, including their susceptibility testing.

Interestingly, despite the intercurrent or recent treatment with approved antimicrobials of all sampled quarters the number of samples yielding a pure culture was high (42.5%). The possible explanations should include too short treatment at the moment of sampling, insufficient mammary gland defence mechanisms to eliminate the pathogen after treatment and existence of resistance on the farm. In discussion with the farm staff it was found that only 4 out of 320 sampled quarters had only a single treatment and most of the 320 quarters had finished treatment in the last 12-72 hours.

A total of 19 out of the 37 CoNS (51.3%) were resistant to antimicrobials

and 14 of them (37.8%) were multi-drug resistant. In total 14/19 CoNS isolates with resistance showed oxacillin resistance.

The 37 CoNS isolates demonstrated no resistance 1, 8 demonstrated resistance to 2 antimicrobials, 5 isolates to a single antimicrobial, 3 isolates to three antimicrobials, 2 isolates to 4 antimicrobials and 1 isolate to 5 antimicrobials.

Conclusions: The results from this study indicate a relatively high prevalence of the multi-drug resistance in CoNS isolates from cases of clinical mastitis on a farm battling with mastitis treatment failure in Australia. The identification of high number of oxacillin-resistant *mecA*-negative CoNS in this study indicate the presence of particular resistance genes not completely proportional to the occurrence of the phenotypic resistance. Thus, it is opinion of the authors that combination of phenotypic and genotypic tests is essential to identify all methicillin-resistant *Staphylococcus* spp.

Anti-Microbial Resistance

P03-003-004

Pharmacokinetics Of A Novel Antimicrobial Peptide In A Ruminant Model

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Objectives: Multi-drug resistance has emerged as a significant challenge to public and veterinary medicine. Use of antibiotics in food animal production is considered a driver of such resistance. Antimicrobial peptides (AMPs) based on innate immune system cationic aromatic peptides could provide an effective alternative to traditional antibiotic use in food animal production. The aim of this pilot study was to determine the pharmacokinetic properties of the antimicrobial peptide TCMCB07 (hereafter B07) following oral, intramuscular and subcutaneous administration in preparation to assess any clinical effects in healthy post weaned dairy calves.

Materials and Methods: This study was designed to advance AMP alternatives to antibiotic use due to the failure to develop new antibiotics to combat multi-drug resistant organisms. The goal was to understand the pharmacodynamics of antimicrobial peptides *in vivo*, to prepare for experiments examining their antibacterial action in food animals, particularly ruminants. B07 was administered to 8 week old Holstein bull calves by oral, subcutaneous (SC), or intramuscular (IM) administration. Blood sampling proceeded for 24 hours after administration. After the initial sampling period, intravenous (IV) doses were given to three animals that received either SC or IM doses, and animals were sampled for another 24 hours. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) was used to measure B07 concentrations in bovine serum samples.

Results: The SC administration of 300 mg of B07 resulted in a 30 min peak of 1.68 ug/ml in blood, while the same dose given IM resulted in a peak of 1.18 ug/ml at 15 min. The SC and IM doses had similar pharmacokinetic curves. A dose of 300 mg of B07 given orally resulted in a peak blood level of 0.11 ug/ml at 24 hours. The low recovery of B07 after oral administration is likely due to extensive degradation of the peptide in the rumen. Two doses, 60 mg and 30 mg, given intravenously resulted peak levels 1 min post administration of 8.96 ug/ml and 1.55 ug/ml, respectively.

Conclusions: The absorption of B07 in circulation was optimized following parenteral administration either subcutaneously or

intramuscularly. Experiment to determine the distribution of B07 in respiratory tissue is currently underway in anticipation of its potential use for treating bovine respiratory disease.

Anti-Microbial Resistance

P03-003-005

Antimicrobial drug use and risk factors for increased treatment incidence and mortality in Swiss veal calves reared under improved welfare conditions

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Objectives: The objectives of the present study were to describe and quantify antimicrobial use and to identify the major risk factors for increased antimicrobial drug use and mortality in veal farms rearing own and purchased calves in Switzerland.

Materials and Methods: A retrospective cross-sectional study was performed. Veal farms producing under a label with improved welfare standards were visited. A pretested standardized visit protocol for the farm characteristic and presence or absence of risk factors was used. Animal data such as birth date, breed, gender etc. were provided by the Swiss national animal movement database. Antimicrobial data was extracted from the farms' veterinary bills and treatment records. Antimicrobial drug treatment incidence was calculated with the defined animal daily dose methodology (TI_{ADD}). Two multivariable models were constructed, for antimicrobial drug treatment incidence (53 farms) and mortality (91 farms).

Results: Ninety-one Swiss veal farms were visited between August and December 2014. The fattening period lasted a mean of 120±28 days, mean carcass weight was 125 kg. Mean herd size was 58±12 fattened calves per year, and purchased calves were bought from a mean of 20±17 farms of origin. The mean TI_{ADD} was 21±15 daily doses per calf and year. The mean mortality risk was 4.1%, calves died at a mean age of 94±50 days, and the main causes of death were bovine respiratory disease (BRD, 50%) and gastro-intestinal disease (33%). No quarantine, shared air space for several groups of calves, and no clinical examination upon arrival at the farm were associated with increased TI_{ADD}. Maximal group size and weight differences >100 kg within a group were associated with increased mortality risk, while vaccination and beef breed were associated with decreased mortality risk. Most antimicrobial treatments (84.6%) were given as group therapy with oral powder fed through an automatic milk feeding system. Combination products containing chlortetracycline with tylosin and sulfadimidine or with spiramycin were used for 54.9%, and amoxicillin for 43.7% of the oral group treatments. The main indication for individual treatment was BRD (73% of the cases). The mean age at the time of treatment was 51 days. Individual treatments were applied through injections in 88.5% of the cases: fluoroquinolones were administration in 38.3%, penicillins in 25.6%, macrolides in 13.1%, tetracyclines in 12.0%, 3rd and 4th generation cephalosporins in 4.7%, and florfenicol in 3.9% of the cases.

Conclusions: The present study allowed for identifying the main risk factors for increased antimicrobial drug use and mortality. This is an important basis for future studies aimed at reducing treatment intensity and mortality in veal farms. These results indicate that improvement is needed in the selection of drugs for the treatment of veal calves according to the principles of prudent use of antibiotics.

Anti-Microbial Resistance

P03-003-006

The dynamics of calves intestinal colonization Extended-spectrum b-lactamase (ESBL)-producing Escherichia coli in terms of dairy farms.

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Objectives: In the last decade antimicrobial resistance monitoring studies have documented rise in the prevalence of resistance to extended-spectrum cephalosporins (ESCs). Resistance to ESCs is often caused by a group of enzymes called extended-spectrum beta (β)-lactamases (ESBLs) and/ or plasmid-mediated AmpC (pAmpC) β – lactamases. In this study we focused on collecting faecal samples from calves since birth to 40 days old.

Materials and Methods: Since birth we were collecting faecal swab and fecal samples. Also swabs from farm environment were taken. Rectal swabs and fecal samples were inoculated directly on MacConkey agar (Oxoid, GB) supplemented with 2 mg/l cefotaxime (Sigma, CR) and incubated 24 hours at 37°C. The fecal, environmental samples (surface smears, in-line milk filters), water, feed (colostrum, milk) and bedding samples were cultured as above with additional pre-enrichment step in MacConkey broth (Oxoid, UK). Simultaneously, the CREC isolates were subjected to antibiotic susceptibility testing by disk diffusion method on Mueller-Hinton agar (Oxoid) in accordance with CLSI (2013a,b) using 12 antibacterial substances: amoxicillin-clavulanic acid (AMC, 30 mg), ampicillin (AMP, 10 mg), cephalothin (KF, 30 mg), ceftazidim (CAZ, 30 mg), chloramphenicol (C, 30 mg), ciprofloxacin (CIP, 5 mg), gentamicin (GN, 10 mg), nalidixic acid (NA, 30 mg), streptomycin (S, 10 mg), trimethoprim-sulfamethoxazole (SXT, 25 mg), sulfonamides compounds (S3, 300 mg), and tetracycline (TE, 30 mg) (Oxoid, UK).

Results: Ninety-six faecal samples were collected from 12 calves 1 to 40 days of age. After cultivation on McConkey agar plates containing cefotaxime (2 mg/litre) cefotaxime-resistant and lactose-positive colonies were counted. The largest increase of colonies was during the third and the seventh day of life. Noticeable drop in AmpCPEC density or its discontinued shedding were recorded in the end of investigated period. All fecal samples from mother-cows, water supply, feed of calves (colostrum, milk, milking parlor) and bedding samples were negative. Positive samples were found on in-line milk filters.

Conclusions: AmpC-producing *E. coli* (AmpCPEC) were detected in faeces of all 12 calves. Shedding density of AmpC-producing *E. coli* has highest concentration 8.0 log₁₀ CFU/gram. It has increasing trend first week of life, than between first and second week the concentration established and downward trend was recorded after the third week of life.

Comments: The paper is based on the IGA VFU Brno project

Anti-Microbial Resistance

P03-003-007

Enrofloxacin resistance monitoring: Antimicrobial susceptibility of bovine respiratory pathogens in Germany

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Objectives: Antimicrobial resistance is a concern in the antimicrobial therapy of both humans and animals. Knowledge on the actual susceptibility and its development over the years is important for ensuring long-term antimicrobial efficacy. Therefore, in the early nineties of the past century Bayer has established a susceptibility monitoring program for target animal pathogens collected from food-producing animals in Germany. Here, the susceptibility status for enrofloxacin (the active ingredient of Baytril®) is presented with regard to bovine pathogens recovered from respiratory tract samples.

Materials and Methods: Samples from diseased animals were collected across Germany between 2006 and 2015. Per farm and outbreak only one isolate was included. The Minimum Inhibitory Concentrations (MICs) of enrofloxacin were determined by agar dilution methodology according to the Clinical and Laboratory Standards Institute (CLSI; VET01-A4, 2013 and previous versions). MIC range, MIC₅₀ (≥5 isolates/species) and MIC₉₀ (≥10 isolates/species) were determined. Enrofloxacin resistance was calculated using the CLSI clinical breakpoint of ≥2 µg/mL for *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* (VET01-S, 2015).

Results: In total, 216 isolates were tested. The most common species isolated from respiratory tract samples were *P. multocida* (n=97) and *M. haemolytica* (n=57). In addition, other species were found in lower numbers, e.g. *Pseudomonas aeruginosa* (n=20), *Trueperella pyogenes* (n=15), *Klebsiella* spp. (n=8), and *H. somni* (n=5). MIC₅₀ and where applicable MIC₉₀ values were 0.008/0.015 for *P. multocida*, 0.03/0.5 for *M. haemolytica*, 1/2 for *P. aeruginosa*, 1/8 for *T. pyogenes*, 0.006/NA for *Klebsiella* spp. and 0.03/NA for *H. somni*. For *P. aeruginosa*, *T. pyogenes* and *Klebsiella* spp. resistance rates could not be determined due to the lack of a clinical breakpoint. For *P. multocida*, *M. haemolytica* and *H. somni* no resistant isolates were found.

Conclusions: This survey demonstrates a very high susceptibility of respiratory pathogens obtained from German cattle to enrofloxacin even after more than two decades of therapeutic use of fluoroquinolones in veterinary medicine. Fluoroquinolone resistance was absent for *M. haemolytica*, *P. multocida* and *H. somni* during the past 10 years. Results are consistent with findings of other national and European monitoring surveys such as GERM-Vet (2012, 2015) and VetPath (El Garch et al., 2015). In spite of this high susceptibility, prudent and responsible use of fluoroquinolones as well as resistance monitoring are imperative.

Anti-Microbial Resistance

P03-003-008

Understanding current practice in the use of antimicrobials in livestock farming and informing interventions to reduce antimicrobial resistance

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Objectives: This research sought to:

- provide evidence-based analysis of how and why antimicrobials (AMs) are prescribed and used in livestock systems,
- explore the extent to which farmers, veterinarians and key actors are aware of the issue of antimicrobial resistance (AMR),
- identify how awareness becomes embedded in and through social

positions and social practice,

- inform future AM interventions in the livestock sector.

Current AM uses in livestock were investigated, focusing upon the interfaces between farming, food production, veterinary and advisory practices as a basis for understanding how changes to these practices might be achieved.

Materials and Methods: A combination of three different yet inter-linked methodological approaches were used:

- A Rapid Evidence Assessment (REA) was undertaken, drawing upon an initial database search of over 4,500 published and unpublished works, narrowed down to 31 papers from which a detailed comparative assessment was generated. The REA also served to contextualise the subsequent empirical research.
- A Q-sort and analysis was developed as a means to interrogate view points and value positions with respect to AM use and related issues (such as animal welfare, public health and governance).
- Qualitative, semi-structured interviews with selected farmers, veterinarians and key actors across the poultry, pig and dairy livestock sectors were undertaken. For each sector, five farmers (at least one of which in each sector was organic) and three vets were interviewed (on farm or in practice). Additionally, interviews were held with a number of key actors from the professional bodies, the food sector and assurance/certification bodies. In total, 32 individual interviews were undertaken. The transcribed interviews were subsequently analysed for common and contradictory themes, for specific examples of practice, for relevant statements that would support identified common positions and for an understanding of the range of practices within which the use and prescription of AMs constituted a regular (or irregular) component.

The REA, Q-sort and qualitative research were then brought together to draw up a series of specific policy-informing messages.

Results: Four evidence categories emerged from the REA: on the volumes/dosage and application of AMs in farming systems, on vet prescription practice in different countries, on farmer attitudes towards AM use and on questions of data and recording of AM use.

The Q-sort suggested there were significant shared values as well as differences across the livestock sectors and across personnel and professions. Shared positions included a relative disdain for the risks posed by farming to AMR in the wider environment and a tendency to underplay the importance of the livestock sector's contribution to AMR as a human health issue. There were three broad positions that vets and farmers tended to take:

- The Modern Business approach - antibiotics are already being phased out through modern farming methods which prioritise hygiene, health planning and herd management; AM use is already appropriate and under control.
- The Interventionist - changes in AM use can be brought about through better farmer education and a veterinary-led understanding of the issue. This position is most amenable to making changes in AM use in livestock.
- The Autonomist - there is a fear that over-zealous regulation or outside influence will lead to inappropriate restrictions and further erode the viability of farming.

These responses suggest the best hope for behavioural change towards more appropriate use is to work with farmers and vets rather than regulate in a top-down fashion.

The qualitative research, by focusing on key actors and allowing them to drive the discussion, enabled deeper insight into these positions.

Conclusions: AMs are seen as a tool in maintaining health of farm

animals - the key issue is their inappropriate use. Principal areas of concern:

- prophylactic and metaphylactic use
- use to treat conditions not caused by sensitive microorganisms
- unnecessary use of later generation AMs
- over-dependence on medicines as a replacement for health management, and
- incorrect dosage

Targeting these areas is a priority, though achieving change requires more than awareness. Changes can be both facilitated and constrained by existing social and practical relationships. Governance of AMs will require a mixture of regulation and responsible action by the industry.

Anti-Microbial Resistance

P03-003-009

A Survey of Anti-microbial Resistance in Organisms Recovered from Sand, Sawdust and Recycled Manure Solids after Use as Dairy Cow Bedding

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Objectives: One concern surrounding the use of recycled manure solids as bedding is that the potential for the closed loop offered by this process may be conducive to the persistence and perpetuation of anti-microbial resistance (AMR) (Bradley et al., 2014). The aim of this study was to establish a 'baseline' level of AMR data and isolates from farms bedding on different materials for the purposes of initial and future comparison.

Materials and Methods: The coliforms and *Enterococcus* spp were selected as organisms likely to be representative of environmental organisms with a known ability to harbour and transfer antimicrobial resistance. Six of each of the selected pathogens were collected from bulk milk (3) and bedding (3) samples from 125 farms using sand, sawdust or RMS as bedding materials. Data on antibiotic use in adult dairy cattle alongside other pertinent management information was collated from questionnaires completed during the farm visits which were conducted as part of a larger survey of bedding use and animal health in the UK.

Antimicrobial sensitivities were determined using a VITEK® 2 (Biomerieux; Basingstoke UK) following the recommended protocols. For the purposes of analysis, minimum inhibitory concentrations (MICs) of each antimicrobial for each organism were ranked and differences analysed using a non-parametric approach. Where univariable analysis identified differences in MIC between bedding types, multivariable analysis was also conducted using conventional models. These models took into account a number of different farm variables and management factors, these included (amongst others) class and route of antibiotic use, geographic location of the farm, organism 'group', housing variables as well as aspects of RMS use.

Results: A total of 724 coliforms and 793 *Enterococcus* spp were collated from 125 farms. Univariable analysis identified significant differences in the MICs of coliforms between the different bedding types for ampicillin (p=0.009), amikacin (p=0.028), chloramphenicol (p=0.049), ceftiofur (p<0.001), cephalixin (p=0.010), enrofloxacin (p=0.003),

nitrofurantoin (p=0.053) and piperacillin (p=0.004). Multivariable analysis confirmed that the difference in MICs between bedding groups could be explained by the farm variables recorded, for ampicillin, nitrofurantoin and piperacillin. Differences were confirmed amongst the other antimicrobials tested. MICs were significantly higher for amikacin for coliforms from farms bedded on RMS compared to sand or sawdust and significantly lower for chloramphenicol for coliforms from farms bedded on RMS compared to sand or sawdust. MICs were significantly higher for enrofloxacin for coliforms from farms bedded on sand compared to RMS or sawdust and significantly lower for ceftiofur and cephalixin for coliforms from farms bedded on sand compared to RMS or sawdust.

Univariable analysis identified significant differences in the MICs of *Enterococcus* spp between the different bedding types for clindamycin (p<0.001) and enrofloxacin (p=0.013); these were confirmed by multivariable analysis. MICs for clindamycin were significantly higher for *Enterococcus* spp recovered from farms bedded on RMS compared to sand or sawdust. MICs for enrofloxacin were significantly higher for *Enterococcus* spp recovered from farms bedded on sawdust compared to RMS or sand.

Conclusions: This study has not generated any clear evidence that the short term use of recycled manure solids as bedding, as compared to sawdust and sand, is associated with a general increase in MICs of the major classes of antibiotics when considering coliforms and *Enterococcus* spp. Further research, using the dataset generated by this study and elsewhere, is needed to further our understanding of any potential interactions between bedding type and management and antimicrobial resistance in the environment.

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Anti-Microbial Resistance

P03-003-010

A Benchmarking Review Of Antibiotic Use In New Zealand From 2005 To 2014

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Objectives: Antimicrobial resistance is widely considered a significant global threat to the health of humans and animals, and the very future of antibiotics in successful animal and human health management is at risk. The New Zealand Veterinary Association has released an aspirational statement that 'by 2030, NZ Inc will not need antibiotics for the maintenance of the health and welfare of animals'. In the context of that goal, it is necessary to determine the current level of antibiotic use in New Zealand. This is the first assessment of antimicrobial use (using sales as a proxy) by population corrected unit (PCU) at a national level in New Zealand.

Materials and Methods: National antimicrobial sales data were available for the past 10 years. Animal population data was available from agricultural censuses, with the exception of wildlife, feral species and minor species such as llamas, alpacas, etc). The total biomass in New Zealand of each species was calculated using accepted industry estimates of bodyweight and age distribution, and the level of antibiotic use was determined based on population corrected units (PCU). Comparative data were compiled from other countries (where available) to enable a comparison between countries of use levels. Finally, these data were compared to the data similarly obtained on the use of antimicrobials in humans in New Zealand.

Results: There has been a slight increase in antimicrobial sales of approximately 2.5%/year or 1.5 t/year over the past 10 years, mostly in the past five years. This was despite a gradual reduction in animal biomass of approximately 4% over that time period. The dairy industry contributed the greatest proportion of biomass to the country total. In comparison with 29 other developed countries, using data for 2012 the last year available, New Zealand was the third lowest user (9.4 mg ai/PCU) of antibiotics in animals. However, this rate of use, which has some annual fluctuations, has increased by approximately 25%. Analysis shows that usage of antibiotics for human health in New Zealand is significantly greater (x12) than for animals although the human use is close to the international average.

Conclusions: This is the first report of baseline data with which the New Zealand animal health industry, especially veterinarians as regulated prescribers, can develop and measure success in maximising the life of antibiotics for animal health and welfare.

In particular, by allowing comparison with international data, these data allow the veterinary profession to move forward on its aspirational goal with a clear understanding of the current position.

Anti-Microbial Resistance

P03-003-011

Molecular Investigation and Antimicrobial Susceptibility of *Staphylococcus aureus* in Mastitic Cholistani Cattle

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Objectives: Sub clinical mastitis primarily with microbial etiology is amenable to different antibiotic therapy and the outcome is doubtful due to association of a variety of microorganisms, host-specificity and continual evolution of different antibiotic resistant strains of microorganisms. Therefore, the present study was ascertained to investigate the presence, genotypic characteristics of *Staphylococcus aureus* isolates recovered from subclinically mastitic cholistani cattle and the effectiveness of different antibiotics against these pathogens by disc diffusion technique

Materials and Methods: For this purpose milk samples were collected from a total of 1457 lactating cholistani cattle and screened for mastitis using California Mastitis Test. Lactating Cholistani cows through cluster sampling were included from 71 villages and 27 tobas of Cholistan in this study. All the positive samples were processed for culturing

of *Staphylococcus aureus* isolates. *Staphylococcus aureus* isolates were identified on the basis of colony characteristics, coagulase test, biochemical features and amplification of *spa* (*spa-X*) and coagulase (*coa*) genes.

Results: The results of PCR revealed that amplification of *spa* (*spa-X*) gene yielded different PCR products (400bp and 350bp) while coagulase (*coa*) produced different products size (390bp, 500bp and 600bp) indicating genetic variation within and among different herds of the cattle. Moreover, results of this study showed that the *spa* (*spa-X*) gene present in coagulase positive (179) and coagulase negative (4) *S. aureus* isolates. *S. aureus* isolates were fully sensitive (88%) to amoxicillin, followed by enrofloxacin (78%) and highly resistant to penicillin (65%) and cephradine (100%)

Conclusions: It is therefore concluded that *S. aureus* isolates were genetically different in the study areas and among the different herds of the animals. In addition the results of this study indicated that the amoxicillin is drug of choice for treating subclinical mastitis.

Anti-Microbial Resistance

P03-003-012

Bacterial Etiology Of Clinical And Subclinical Mastitis In Indigenous Goats And Multiple Drug Resistance Of The Isolates

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Objectives: Due to the aforementioned economic and sanitary problems, the research will be to isolate and identify the pathogens that are causing intramammary infection in small ruminants in herds in communal areas of Mafikeng. Following identification, the pathogens will be evaluated for their antibiotics susceptibility profile to suggest suitable antibiotic treatment

Materials and Methods: Isolates were Gram stained using standard techniques, which differentiates bacterial species into Gram positive and Gram negative (Cruikshank et al., 1975). A number of classical phenotypic tests were performed using BioMérieux API 20E system. The total genomic DNA of cultivated isolates was purified following Zymo-Research Fungal/Bacterial DNA kit instructions. The concentrations of purification of DNA were assessed using a nanodrop (Bio-Rad, South Africa). Amplification of 16S rDNA gene was carried out in reaction volume of 50 µL. Two sets of primers were used: forward 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse 1492R (5'-TGA CTG ACT GAG GCT ACC TG-3'). Amplified fragments of DNA were resolved in 1% w/v agarose gel and visualized using Gel Doc TM XR+ with image Lab TM software (Bio-Rad, South Africa) to confirm the expected size of the product. The isolates sequences will be subjected to BLAST searches to verify identity, taxonomic classification based on sequence similarity measures, and phylogenetic interpretation. Five antibiotic discs (Oxoid) were used: Gentamicin (10 µg), Streptomycin (10 µg), amoxicillin (5 µg), Ciprofloxacin (5 µg) and Ampicillin (10 µg). The tests were performed according to the guidelines of the Clinical Laboratory Standards Institute (Bauer et al., 1996)

Results: A total of 1000 raw milk samples from goats were analyzed. Samples were obtained from different collecting points from Mafikeng town; North-South. The prevalence of clinical mastitis was found significantly ($p < 0.01$) higher in South ($H = 47$; 79.66%) in comparison to the North ($H = 12$; 20.34%). Bacteriological analysis showed that South

had 94.44% of bacterial infection compare to North 15%. No significant differences was observed on the status of bacterial pathogens between clinically (H = 55; 93.22%) and sub-clinically (H = 47; 95.92%) of both areas. The major bacterial isolated were *Staphylococcus* spp. (38.98%), *Escherichia coli* (27.12%) and *Bacillus* spp. (10.17%). Antibiotic susceptibility results showed a relatively high level of resistance to ampicillin, amoxicillin and streptomycin, whereas gentamicin and ciprofloxacin were found to be the most effective drugs

Conclusions: These results indicate the high prevalence of mastitis in apparently normal quarters of lactating goats and therefore, there is a need for the prudent use of antibacterial in animal health and production through bacteriological and antibiogram studies and therefore, there is a need for the prudent use of antibacterial in animal health and production through bacteriological and antibiogram studies in mafikeng (South Africa).

Anti-Microbial Resistance

P03-003-013

Assessing the prevalence of ESBL *Escherichia coli* and antimicrobial resistance of *E. coli* isolates in cattle faeces on farms in N. Somerset, England.

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Objectives: The first aim of this project was to ascertain the prevalence of antimicrobial resistance (AMR) including multidrug and extended-spectrum beta lactamase (ESBL) resistance in *E. coli* isolates (i.e. the proportion of *E. coli* isolates showing resistance to one or more antimicrobial). These isolates were from adult dairy cattle faecal samples on North Somerset farms, England, which have reduced their use of critically important antimicrobials over the last five years.

The second aim was to examine the correlation between the amount and type of antibiotics used on these farms and the prevalence of antimicrobial resistance AMR in *E. coli* isolates.

Materials and Methods: Cattle were enrolled prospectively on seven conventional dairy herds in North Somerset which varied in size between 100 and 200 milking cows. Faecal samples were collected from rectal gloves after palpation of cows presented to veterinary surgeons for fertility examinations. Information about animal numbers and management practices was collected for risk factor analysis. Kirby-Bauer antibiograms were performed for each isolate (bsac.org.uk). These antibiograms were designed to look for patterns of resistance associated with plasmids that also carry ESBL resistance, as well as representing the antimicrobial groups commonly used on farms. Resistant isolates (those showing resistance to one or more antimicrobial) were stored for future pulsotyping, and all faecal samples were stored for use in future research.

Medicine audits, performed using medicine sales records from the University Farm Animal Veterinary Practice for the previous four years, were available for all farms. These audits were used to assess the antimicrobial use on each farm. Medicine sales records were also used to calculate animal daily doses of each group of antimicrobials prescribed and supplied to each farm.

Correlation between antimicrobial use and the presence of AMR, including ESBL, and risk factors for AMR are being assessed using

logistic regression, and the presence of resistant isolates will be analysed with respect to antimicrobial usage on each individual farm over the previous four years.

Results: Preliminary results are presented as only half (approximately 700) isolates have so far been processed and are available for analysis. These initial isolates show the highest levels of resistance to amoxicillin (mean 86%, range 79–96%), streptomycin (mean 29%, range 0–58%) and amoxicillin-clavulanate (mean 5%, range 0–9%). Resistance was present on at least one of the seven farms for every antibiotic group tested, other than the carbapenems (imipenem).

Multidrug resistance (resistance to 3 or more antimicrobials) was found on all seven of the farms. The mean prevalence of multidrug resistance was 6.7% (6.5 isolates), with a range from 2–9% (1–15 isolates) between farms. Multidrug resistant isolates are primarily resistant to many, if not all, of the following antimicrobials: streptomycin, amoxicillin and amoxicillin-clavulanate.

Resistance to extended-spectrum (3rd and 4th generation) cephalosporins was found on four of the seven farms. Resistance to fluoroquinolones was also found on four of the seven farms, but only one farm had resistance to both fluoroquinolones and extended-spectrum cephalosporins together. Interestingly, this farm had only reduced its use of protected antimicrobials 18 months before the study began, and therefore had used these classes of antimicrobials most recently compared to the other farms.

ESBL *E. coli* isolates were found on three of the seven farms at prevalences of 0.6 – 2.7% (1 – 4 isolates).

Conclusions: The preliminary results of this study suggest that a wide variety of resistance mechanisms are present in faecal *E. coli* from cattle on farms in North Somerset, and that up to 9% of isolates on individual farms, are multi-resistant.

ESBL *E. coli* isolates have also been found on three of the seven farms, confirming that dairy cattle are a reservoir of ESBL isolates in North Somerset. The prevalence of ESBL *E. coli* thus far is lower than has been reported in at least one other study performed on a UK dairy (Watson et al., 2012).

Comments: Further analysis is underway regarding association and correlations between antimicrobial use on the farms and AMR, as well as risk factors for AMR. These analyses should be available for presentation by WBC in July 2016.

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Anti-Microbial Resistance

P03-003-014

Isolation And Characterization Of The Antibacterial Effect Of Bacteriophages Specific For Shiga-Toxic *Escherichia Coli* Strains Isolated From Calves

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Objectives: The growing phenomenon among many bacterial antibiotic resistance and the associated difficulty in effectively eliminate infections contribute to the development of alternative methods for the elimination of pathogens, such as, a phage therapies. Based on these, the aim of the study was the isolation and estimation of antibacterial activity of chosen bacteriophages specific for Shiga positive *Escherichia coli* strains isolated from calves in In vitro conditions.

Materials and Methods: The material consisted of 20 Shiga positive (STEC) of *E.coli* strains isolated from calves. The 12 *E. coli* strains were obtained from the National Veterinary Research Institute in Pulawy (Department of Hygiene of Food of Animal Origin) and the 9 strains were coming from own collection and were isolated from calves with clinical signs of diarrhea in Lublin's region.

Isolation of bacteriophages was carried out from sewage obtained from cattle housing systems according to Huff et al. (2002). Bacteriophage morphology was examined using a transmission electron microscope by negative-stained prepare with 2% silicotungstate (Xie et al. 2005). The range of lytic activity against Shiga-toxic strains of *E. coli* was determined by plaque assay on double top agar plates according to Huff et al. (2002). The lytic titres evaluated by the dilution method in SM buffer according to Golec et al. (2011).

The genetic material from the obtained bacteriophages has been isolated according to the protocol of phage DNA Isolation Kit (NORGEN, Ca). The restriction analysis of the obtained material has been carried out with using of restriction enzymes Hind III, ClaI and XbaI with (Fermentas, Lithuania). The procedure of restriction analysis has been carried out according to the producers's instruction.

Results: Overall, the present study were obtained 8 bacteriophages that based on morphological qualified for two families, five of their belong to Myoviridae and three to Siphoviridae. The lytic titres evaluated by the dilution method in SM buffer were ranged from 5×10^5 pfu/ml to 4×10^{10} pfu/ml add unit. The obtained in the restriction analysis profiles showed a similar restriction fragments ranged from 405.14bp to 4659.75 bp. The obtained results showed that the examined phages specific to ETEC strains isolated from calves. Those phages had complete lysis on *E.coli* strains on double-layer agar plates, and showed an antibacterial activity against 12 examined *E. coli* strains. This result confirms the antibacterial activity of the obtained bacteriophages and is beneficial for using of bacteriophages in elimination of *E. coli* infections in cattle.

Conclusions: To sum up, it can be said that the obtained phages exhibit antibacterial activity against to examined ETEC strains isolated from calves. The obtained results allow for further research in order to increase their use of targeted therapies in calves with symptoms of diarrhea caused by *E. coli*.

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Anti-Microbial Resistance

P03-003-015

Successful treatment of a multi-drug resistant *Pseudomonas aeruginosa* infection following a digit amputation in a Belgian Blue calf.

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Objectives: Digit amputation is a commonly performed surgery in the bovine patient. Whilst it is most commonly performed for infections of the deep structures of the foot, here we describe its application to resolve a chronic proximal interphalangeal septic arthritis.

Materials and Methods: An 8 month-old Belgian Blue calf presented to the Royal (Dick) School of Veterinary Studies with a month long history of right forelimb lameness. The owner of the animal had instigated treatment (without veterinary consultation) with daily intramuscular penicillin & dihydrostreptomycin, which he continued for 1 month without any improvement in the degree of lameness. Radiography revealed complete destruction of the medial proximal interphalangeal joint (PIPj) with lysis and bony proliferation of the proximal aspect of the proximal phalanx (P1). Due to the location, chronicity and severity of the infection, digit amputation by a high approach was indicated.

Upon lack of clinical improvement the pastern was again radiographed and radiographic findings used to guide further curettage of any devitalised tissue. The wound was swabbed for bacteriological culture and sensitivity due to the poor rate of healing, the infected appearance of the developing granulation tissue and the unpleasant smell and greenish discharge associated with the wound.

Results: Multi-drug resistant *Pseudomonas aeruginosa* infection was subsequently diagnosed as the cause of poor healing of the surgical wound.

Antibiotic administration was therefore discontinued and the treatment regime changed to focus on topical wound management. The wound was lavaged daily with 3% Hydrogen Peroxide applied under pressure using a 30ml syringe and 16 gauge needle. Ordinary table honey was applied with a clean dressing daily. This was continued for one week, after which time the wound bed was filled with healthy granulation tissue, only normal physiological discharge was seen on wound dressings and the degree of lameness was markedly improved. Wound dressing was then halted. The wound was covered in silver sulphadiazine cream and left open. The animal was discharged 3 days later.

Conclusions: This case report highlights the benefits of targeted diagnostics (radiography and bacterial culture and sensitivity) in the treatment of refractory or complex orthopaedic cases in cattle and the use of non-pharmacological therapies (hydrogen peroxide and honey dressings) in the treatment of antibiotic resistant pathogens.

Comments:

- Diagnostic imaging can be a useful tool in guiding decision making in orthopaedic procedures in cattle.
- Culture and sensitivity of causative organisms should always be considered as part of responsible use of antimicrobials, particularly when poor treatment responses occur.
- Alternative therapies and wound care can produce excellent results.
- A considered approach to diagnostics and case management are vital to maintain animal welfare and minimise economic losses.

Anti-Microbial Resistance

P03-003-016

How good management practices reduce the curative antibiotic use in cattle: the example of subclinical ketosis

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Objectives: Good herd management is known to mitigate antimicrobial use, but few to no quantification of such a relationship is available. Subclinical ketosis (SCK) promotes the occurrence of infectious diseases which require antibiotics for curative treatment. The control of SCK is based on peripartum management and/or use of preventive monensin bolus (Kexxtone®) for cows at risk for SCK. The objective is to quantify the decrease in curative antibiotic use (i) when farmers reduce the prevalence of cows at risk for SCK or (ii) when they use the bolus Kexxtone® to prevent SCK in cows at risk for SCK.

Materials and Methods: A stochastic model was built to calculate the total quantity of curative antibiotics at the herd level for various prevalences of cows at risk for SCK. Scenarios with or without Kexxtone® to prevent SCK (with fear or good targeting of cows) were done. The prevalence of SCK was determined for (i) the various prevalence of cows at risk for SCK and (ii) using or not Kexxtone® on cows at risk. The total quantity of curative antibiotics used is calculated thanks to the prevalence of SCK and to the odd-ratio of diseases in case of SCK compared to no SCK. Most of these diseases were considered treated with antibiotics, as commonly seen on the field.

Most popular scenarios included: prevalence of cows at risk for SCK between 10 and 80 percent; efficacy of monensin to prevent SCK fixed at 0.45, 0.66 and 0.85; OR for cows at risk for SCK to really have SCK fixed at 2.2 or 4.5. Calibrations were provided by literature review, meta-analysis and expert opinion.

Results: First, the association between the decrease in prevalence of cows at risk for SCK and the decrease in the quantity of curative antibiotics varied between situations. The decrease in the quantity of curative antibiotics started from few percent for low decrease in cows at risk for SCK, but reached 10 to 25 percent in the most popular scenarios (with calibration taken on the average of ranges). For instance, the decrease in the prevalence of cows at risk for SCK from 80 to 10 percent, corresponding to a decrease of SCK prevalence from 68 to 17 percent, led to a decrease in the quantity of curative antibiotics by 25 percent. The relationship was linear. With the extreme calibration, the decrease in quantity of curative antibiotics achieved 35 percent. Whatever the calibration, the low decrease in the quantity of curative antibiotics compared to the decrease in SCK prevalence was in agreement with the base level of curative antibiotics use in cows without SCK.

Second, using monensin with very good to good accuracy (perfect targeting or 80 percent of cows well targeted, respectively) allowed a decrease up to 20-25 percent in the quantity of curative antibiotics when the prevalence of cows at risk for SCK was high. The decrease in quantity of antibiotics allowed when the prevalence of cows at risk for SCK was moderate is from few percent up to 10-15 percent. Results showed that the quantity of curative antibiotics was stabilized at the level observed for low prevalence of cows at risk for SCK, when this prevalence was high and the monensin bolus was used.

Conclusions: The results showed that good management practices such as SCK control thanks to peripartum management (without Kexxtone®) were a powerful way to reduce the curative antibiotic use. The results are of great interest when compared to the 25 percent decrease objective provided by French policy-makers (EcoAntibio program).

Using Kexxtone® is also a powerful tool to stabilize the curative use of antibiotics when the prevalence of cows at risk for SCK is increasing. When Kexxtone® is used, the hazard regarding antimicrobial resistance that is linked to either the use of monensin or curative antibiotics (such as cephalosporin) has now to be assessed.

Anti-Microbial Resistance

P03-003-017

Methods used in a Flemish campaign to create awareness on responsible antimicrobial use in the veal industry

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Objectives: Antimicrobial consumption in Belgian livestock is among the highest of Europe, and this is translated in high levels of antimicrobial resistance in commensal, zoonotic and pathogenic bacteria. Especially in pigs, poultry and veal calves antimicrobial use is intensive. The Flemish government funded a project (the responsible use of antibiotics in intensive reared livestock) with the objective to create awareness in farmers and veterinarians. The objective of this abstract is to describe the topics communicated, and the ways of communication, used to create awareness about antimicrobial use and resistance in the veal industry.

Materials and Methods: An expert panel consisting of academic staff (n= 3), veterinarians of the animal health service (n=2) and veterinarians active in the veal industry (n=5) was constructed and guided by the Flemish advice organ on veterinary antimicrobial use (AMCRA). Available information on administration ways and indications for antimicrobial use in the veal industry was inspected, and possibilities to reduce were listed. Next, actions to reduce antimicrobial were priority ranked according to their possible contribution to antimicrobial reduction, the likeliness to be implemented at short term and the ratio labor/achieved reduction. Subsequently, the campaign was focused on the top 5 action points. At every stage, outcomes were communicated with the persons involved in the same exercise for pigs and poultry.

Results: Ranking according to the expert panel was as follows: (1) avoiding routine antimicrobial group treatments, (2) avoiding two or more antimicrobials in one treatment, (3) calf quality control at arrival, (4) stable ventilation adjustment, (5) correct dosing of antimicrobials, (6) avoiding oral antimicrobial use, (7) avoiding group treatments and (8) vaccination upon arrival. The first two measures were at the veterinarians discretion and had already been markedly adjusted. The third measure, calf quality control at arrival, could only be influenced by the integrators and not by the farmers directly. Parameters for selection (immunoglobulin levels at arrival, a bodyweight over 50 kg at arrival, no persistent infection with bovine viral diarrhoea virus and no diarrhoea or respiratory signs) were nevertheless communicated to the farmers. Ventilation audits were performed in four veal farms to collect practical examples including fumigation tests. The main problems on the farms were hypoventilation and draught. To stimulate correct dosing, heart girth measurements were performed on 698 veal calves (6 herds), and the association with body weight was determined by linear regression. In this way, farmers could more accurately estimate body weight by a simple heart girth measurement. Finally, to what considers individual treatments made by

the farmer, 'avoiding the use of two or more antimicrobials' was made one of the central one-liners in the communication campaign.

Conclusions: Overall the campaign was focused on a correct stable ventilation, correct dosing of a single antimicrobial with the least importance for human medicine and avoiding group treatments. The methods used and the action points identified in this project might aid other countries with similar industries to create awareness about responsible antimicrobial use.

Comments: Special acknowledgement for the important contribution of Magali Blomme to this project

Anti-Microbial Resistance

P03-003-018

Antimicrobial Resistance profiles of bacteria of Medical and Veterinary importance found in the bovine gut and the farm environment

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Objectives: Antimicrobial resistance (AMR) is a threat to modern medicine. Concern has been expressed about the safe use of these drugs and their future efficacy. Although antimicrobial usage on farms was implicated as a potential driver for the selection of resistant bacteria, little is known of pathogen and AMR profiles on UK beef and dairy farms. The project will identify carriage of resistance markers in part of the national herd. The results will have implications for farming practice and public health with the potential of zoonotic spread of the organisms concerned.

Materials and Methods: Farms from South Central England were included in this study, with representative samples taken from both dairy and beef herds. At farm enrolment: history of antimicrobial use; type of farming undertaken; breed; milking and calving status were documented. Environmental samples from the farms were also collected and analysed over the period of the study. Targeted pathogens isolated were analysed to give a farm level assessment and changes were tracked.

Predominantly marker strains of *E. coli*, *Salmonella* sp. *Klebsiella* sp. and *Strep. faecalis* were targeted. The media used included MacConkey agar, Sorbitol-MacConkey agar, Modified Edwards agar, Brilliant green agar and Nutrient agar. Cultured organisms were then identified using a variety of standard biochemical tests to provide a metabolic profile and to identify the organisms present. Where appropriate, identification systems such as API systems were employed.

Once confirmed, the antibiotic efficacy profiles of the isolates were determined. The panel of antibiotics tested using EUCAST methodology were: 30µg cefquinome, 25µg amoxicillin, 10µg ampicillin, 30µg oxytetracycline, 30µg tylosin and 5µg marbofloxacin (antibiotic concentration/disc). The plates were then incubated at 37°C for 20 hours. After incubation the diameter of the zone of clearing around the disks were measured and recorded. Through analysis of the data collected during farm enrolment in combination with the bacteriology, risk factors for development of antimicrobial resistance and training needs were identified.

Results: The bacteria isolated from the initial farm were: *E. coli* (74.3%), *Pseudomonas* sp. (5.9%), *P. vulgaris* (6.7%), *Klebsiella* sp. (1.4%), *Corynebacterium* sp. (1.4%), *P. mirabilis* (1.4%) and *Streptococcus* sp.

(8.1%). Of all the isolates, 63% showed resistance to the antimicrobials in the panel according to the EUCAST guidelines.

Of the bacteria isolated, 31.5% were resistant to ampicillin (AMP), of which 14% were in the bovine gut (BG) and 18% was from environmental samples (ES). A total of 2.4% of isolates were resistant to cefquinome (CEF) (1.6% from the BG and 0.8% from the ES), 1.6% were resistant to marbofloxacin (MAR) (0.8% from both the BG and ES) and 49.2% were resistant to Amoxicillin (AML) (24.2% from the BG and 25% from the ES). Overall 35.5% of the resistance seen was in the BG and 26% was seen from ES. From the *Streptococci* isolated, 33% showed resistance to Tylosin (TY) (2.7% from the BG and 30.3% from the ES).

The *E. coli* isolated showed 22.6% resistance to AMP, 0.8% resistance to CEF and MAR and 33.1% resistance to AML. 2.4% of the *Pseudomonas* sp. were resistant to AMP and 4.8% were resistant to AML. *P. vulgaris* demonstrated resistance of 4.8% to AMP, 0.8% to CEF, 4.8% to AML. *Corynebacterium* sp. and *P. mirabilis* sp. showed resistance only to AML of 1.6%.

Of the bacteria isolated, 18.6% were resistant to both AMP and AML, of these 15.3% were from ES, and 3.3% were from the BG. One *E. coli* isolated from ES showed resistance to MAR as well as AMP and AML and a *P. vulgaris* also from ES showed resistant to CEF as well as AMP and AML.

Conclusions: Preliminary results show that a higher level of resistance was seen in the BG compared to the ES, however, multi-resistant isolates were more commonly seen in the ES. High levels of resistance were seen against AMP and AML. This is of concern, as they are used in the treatment of human infections. No clinical breakpoints were found for CEF, MAR and TY, so experimental breakpoints were used for these initial samples. The lack of clinical breakpoints for these drugs show that more research is needed to depict resistance in veterinary medicine. This information will help evaluate the level of antibiotic resistance on beef and dairy farms.

Anti-Microbial Resistance

P03-003-019

Impact of enrofloxacin dosing regimens in cattle on intestinal pharmacokinetics and the selection for resistant enteric bacteria

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Objectives: The objective of this study was determine the impact of two FDA-approved dosing regimens of enrofloxacin on the active drug concentrations in the gastrointestinal tract, and to associate these concentrations with changes in the fecal bacteria of the steers.

Materials and Methods: Ultrafiltration probes were surgically implanted into the ileum and spiral colon of 12 6-month old Holstein steers. In a cross-over study, each steer was administered enrofloxacin either once (12.5 mg/kg subcutaneously) or once a day (5 mg/kg subcutaneously) for 3 days. After the sampling period and a 1 week washout, steers were reassigned to the other treatment group. Plasma, interstitial fluid, and intestinal fluid were collected during treatment and for 72 hours after the last dose for pharmacokinetic modeling. Fecal samples were collected

every 12 to 24 hours during treatment and for 7 days after the last dose. Fecal samples were collected twice a week from a subset of the calves for 1 month after the last treatment. Samples were cultured on selective media to quantify *E. coli* and *Campylobacter* spp. Ten isolates from each time point were further characterized by microboth dilution to determine the minimum inhibitory content (MIC) of enrofloxacin.

Results: Fecal *E. coli* concentrations decreased significantly immediately after treatment in both groups, but returned to baseline by 72 hr in the high dose group and 144 hr in the low dose group. The impact of dosing on *E. coli* MIC was dependent on treatment order. In the steers that received the high dose first, the median *E. coli* MIC did not change after the high or low dose. In the steers administered the low dose first, the median *E. coli* MIC increased significantly in the low dose group from time 0 to day 8, but returned to baseline by day 12. At that time, they received the high dose, and the MIC again increased significantly at day 7. All animals were positive for enrofloxacin-susceptible *Campylobacter jejuni* either by direct plating or by enrichment (or both). *Campylobacter* could not be recovered for the first 24-120h after treatment, but samples from 144-196h post-treatment were again *Campylobacter*-positive, suggesting that the populations of the organism were temporarily reduced below detection levels in response to treatment. Pharmacokinetic analysis is pending.

Conclusions: Administration of enrofloxacin by either FDA-approved dosing regimen appears to cause significant, but short-lived changes in the fecal bacteria of cattle. Associating these changes with the pharmacokinetic parameters may allow for better risk assessment models to predict the impact of systemic antimicrobial administration on shedding of antimicrobial resistant pathogens in cattle.

Anti-Microbial Resistance

P03-003-020

Antimicrobial Susceptibility Of Staphylococci Strain Isolated From Milk Of Goats Reception Pot With Mastitis The Municipality Of Tanhuato Michoacan

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Objectives: The aim of this study was to determine the antimicrobial susceptibility of staphylococci isolated from milk of goats receiving pot municipality Tanhuato, Michoacán.

To evaluate the antimicrobial resistance of *Staphylococcus aureus* strains isolated from milk of goats boat reception Tanhuato municipality, Michoacan.

Materials and Methods: The present study was conducted from May 25, 2014 to December 17, 2014, lasting 206 days. The study was carried out with 49 flocks of which an equal number of samples taken. Sampling of milk is performed using for this test tubes sterilized sealing plug in which the necessary identification previously stable are scored. The test tube was placed inclined to keep out dirt. The sampling was made directly from the milk storage tanks. The sample tube is filled with at

most two thirds. Finally the tubes were placed with the sample in a rack placed in a sealed cooler and then are transported to the laboratory of bacteriology of USAD (Unit Auxiliary Services for Diagnosis), Faculty of Veterinary Medicine and Animal Husbandry for cooling to 4 ° C and then for processing.

Identification of Staphylococci was conducted through its colony morphology, Catalase test, Gram staining, Coagulase test, test Mannitol, Gelatin and Hemolysis. The antimicrobial susceptibility testing was performed through the disk diffusion method on Mueller-Hinton agar. Staphylococcus were isolated in 29 herds in the town of Tanhuato, Michoacán

Results: Of which 21 correspond to *Staphylococcus aureus* (72.41%) and 8 developments (27.58%) Coagulase Negative Staphylococci to (ECN) (*Staphylococcus epidermidis*). In the case of *Staphylococcus aureus* was observed increased resistance against Penicillin, Sulfamethoxazole, Ampicillin and Erythromycin with 42.9%, 38.1%, 38.1%, 38.1% respectively, followed by 23.9% to Gentamicin, 19.1% Ceftazidime and Cefotaxime. Regarding the susceptibility observed in 90.4% to Cephalothin, Tetracycline and Cefuroxime, Pefloxacin and followed by 85.7% Doxycycline. In the case of ECN greater resistance to Trimethoprim was observed with 50%, 37.5% Doxycycline, Erythromycin, Ampicillin and Gentamicin with 25.0% each, whereas the more susceptible for ECN were Tetracycline, Penicillin and Cephalothin with 100%, and Pefloxacin, Cefotaxime, Cefuroxime, Ceftazidime with 87.5%.

Conclusions: It is concluded that the isolates of *S. aureus* and *S. epidermidis* (ECN), milk pot from reception, show different patterns of antimicrobial resistance, closely related in some cases with little chance of exposing animals in production to antimicrobial therapy, reducing bacterial selection pressure .

BVD

P03-003-022

Role of imported ruminants in the genetic heterogeneity of pestivirus strains in Northern Ireland

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Objectives: The genus pestivirus within the family Flaviviridae includes bovine viral diarrhoea virus (BVDV) types 1 and 2, border disease virus (BDV) and classical swine fever virus. The two recognised genotypes of BVDV are divided into subtypes based on phylogenetic analysis. The genetic heterogeneity of pestiviruses from clinical samples collected in Northern Ireland between 2008 and 2011 was investigated and compared to those previously described in Northern Ireland and the Republic of Ireland. In addition, the role of cattle and sheep importation in expanding the genetic diversity of these isolates was explored.

Materials and Methods: 91 BVDV positive samples from 88 herds submitted to AFBI between 2008 and 2011 were selected for further sequencing. In addition, RNA from samples from 839 bovine and 4,437 ovine animals introduced to the region during 2010 and 2011 were tested for the presence of pestiviral RNA with an reverse-transmission polymerase chain reaction (RT-PCR) assay (VetMAX Gold BVDV, Life Technologies) and the positive samples selected for sequencing. A 288 base pair portion of the 5' untranslated (UTR) region was amplified by RT-PCR and the product sequenced. The sequences were aligned and compared with the corresponding sequences of a number of reference strains and with 25 previously reported sequences.

Results: The analysis indicated that the predominant subtype circulating between 1999 and 2010 was BVDV-1a (86 samples out of 91, 94.5%). Five out of the 91 samples clustered close to reference strains in subtype BVDV-1b (5.5%). 18 out of the 839 samples from bovine animals introduced to Northern Ireland gave a positive result (2.14%, Ct<37) and 8 an inconclusive result (0.9%, Ct ≥37) comprising a total of 24 animals. A true prevalence of BVD virus positive bovine animals within those introduced was calculated as 2.9% (1.7-4%). Sequence data were obtained for eight of these samples, all of which clustered close to reference strains in subtype BVDV-1b. No positive results were obtained with the ovine samples.

Conclusions: Since only the BVDV-1a subtype was detected in samples collected between 1968 and 1999, the present study suggests that at least one new subtype (BVDV-1b) has been introduced to Northern Ireland between 1999 and 2010. BVDV-1b was also the only strain detected in imported cattle. This finding highlights the potential for the importation of cattle to introduce new strains. In contrast, the risk associated with the introduction of sheep was found to be minimal.

BVD

P03-003-023

The confirmation of the presence of Bovine Viral Diarrhea type 2 in Belgium.

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Objectives: Bovine Viral Diarrhea Virus (BVDV) is often unexpectedly present in herds. Two genotypes exist: BVDV type 1 and BVDV type 2. In the USA and Canada about 45 % of the genotyped BVD strains belong to the BVDV type 2. In Europe the prevalence of type 2 is estimated at 5 %. Since the first of January 2015 an official BVD eradication program was started in Belgium. In a further evolution of BVD eradication cattle becomes more vulnerable in case of renewed virus contact. Protection on herd level by means of vaccination could prove to be a valuable tool. Therefore it is essential to know if BVDV type 2 is present.

Materials and Methods: In Belgium veterinary practitioners send in samples for diagnosis and monitoring to two official labs (DGZ - Diergezondheidszorg and ARSIA- Association regional de santé et identification d'animaux). In case of BVD analysis those labs mainly use two tests to look for BVD antigen, a BVD Ag ELISA test and a BVD Ag PCR. Analyses were carried out on different matrices. These tests however cannot make the difference between BVDV type 1 and BVDV type 2. The reference lab: CODA-Cerva, disposes of a BVD Ag PCR test which on the other hand can distinguish between BVD type 1 and BVD type 2. In order to obtain samples for further analysis two approaches were taken. First the veterinary practitioners were asked to send in samples when they had a suspicion of BVD, secondly the two official labs were asked to send all their positive BVD Ag samples to the reference lab for typing. Every matrix was analyzed except for ear notch samples. For the majority of these latter samples we don't have any information on the motives for which these samples were analyzed in the first place. This search was performed in the period between August 2014 and April 2015.

Results: Following the first approach 21 samples were sent in by veterinary practitioners. In these cases there was clearly a suspicion of BVD infection, either by clinical symptoms or by a formal positive spot test i.e. antibody search in young stock to determine virus circulation. Four of these samples were positive for BVD type 2. Two samples originated from two different animals on the same farm; in this case it concerned two confirmed PI (persistently infected) animals. The lab of DGZ in the northern part of the country has sent 113 samples to the CODA-Cerva (the reference lab). Three of those samples were positive for BVD type 2. The lab ARSIA in the southern part of the country has sent 104 samples to CODA-Cerva. Again three samples were positive for BVD type 2. In case there is already a suspicion by the vet of BVD infection up to 19 % of the samples reacted positive for BVD type 2. It concerns a limited number of samples in a limited period of time. On the other hand, samples sent by the labs DGZ and ARSIA to the lab of reference (CODA-Cerva) 2,76 % were found positive for BVD type 2. In the given period 9 samples were found positive on 8 different farms on a total of 221 samples. This results in 4 % of the samples being positive for BVD type 2. All analyzed samples originated from animals from over the country. Positive samples were traced back to different parts of the country but with a small concentration of some positive samples in the eastern part of the country.

Conclusions: The presence of BVDV type 2 is confirmed in Belgium and matches with the estimated presence of BVD type 2 in Europe. The second approach has shown that BVD type 2 is present without expecting it, since no motive was known for analyzing these samples. This unknown presence of the virus can cause hidden loss on farm level. If the vet has the suspicion of a BVD infection there is a substantial possibility BVD type 2 is present. Fourthly, when in a further evolution of an eradication program protection by vaccination is discussed one needs to be aware that it is necessary to include both types BVD in the protection.

Comments: The author was veterinary practitioner in a cattle practice for 20 years. Then he worked for three years at an Animal health service organization in Belgium. Since April 14, 2014 he joined Boehringer-Ingelheim as Field Technical Manager Cattle & Horse.

BVD

P03-003-024

Retrospective evaluation of herd health data shows that calf mortality could be an indicator for BVDV infection.

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Objectives: Our objective was to examine whether herd health data can be used as an indicator or early warning for Bovine Virus Diarrhea virus (BVDV) circulation. Our goal is to provide the veterinary practitioner with an easy tool to be able to advise the farmer to take timely measurements on BVD.

Materials and Methods: In the Netherlands continuous animal health monitoring (CAHM) data is part of the mandatory herd held management by the vet. The vet has access to this data. Retrospective evaluation of herd CAHM data on farms with a history of BVDV circulation was performed to analyze if calf mortality could be an indicator of BVDV infection in a herd.

Three farms with a history of being BVD virus free (period 1), followed by a period of BVDV circulation in 2014 (period 2) and becoming BVDV free again (period 3) were analyzed. Retrospective data of the 3 periods per farm were evaluated and health parameters such as calf mortality were calculated. The calf mortality was also compared to the Dutch national average and the data from BVDV free farms (n=38) in the same practice. Calf mortality is defined as the number of dead calves less than one year of age divided by the total number of calves less than one year old. Data from the rendering organization and the national identification and registration system is used.

Results: During the period of Bovine Virus Diarrhea virus circulation all three farms showed an increase in calf mortality (5,75) compared to the period before (0,54) and after (2,25). Also compared to the Dutch national average calf mortality of 3,23 calves in the same year (2014), the calf mortality on farms with Bovine Virus Diarrhea virus circulation was increased.

The average calf mortality of 2,57 on the Bovine Virus Diarrhea virus free herds in the same practice was lower than the Dutch national average.

Conclusions: The results shows that calf mortality seems to be lower on BVDV free farms and higher on farms with BVDV circulation. Herd health data, such as calf mortality, can be used as indication for BVDV circulation. This data can be monitored by the vet even without being on the farm. The vet has an extra tool to advise the farmer to take timely measurements on BVD.

BVD

P03-003-025

Variability Of Bovine Viral Diarrhea Virus In Type 1A Persistently Infected Calves

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Objectives: Bovine viral diarrhoea virus (BVDV), a Pestivirus in the family Flaviviridae, is an economically important pathogen of cattle worldwide. The primary propagators of the virus are immunotolerant persistently infected (PI) cattle, which shed large quantities of virus throughout life. However, up to now very little was known regarding the viral population structure in these animals. The goal of this study was to describe the extent and distribution of BVDV variability in multiple body compartments of naturally infected PI cattle derived from a Western Canadian beef herd using next generation sequencing techniques.

Materials and Methods: A cow-calf herd with a no vaccination policy experienced an introduction of BVDV in their BVDV naïve herd during, or just shortly following the 2013 breeding season. This season is 2 months during summer as is common in Alberta. Due to this short timeframe the fetuses were in approximately the same state of their development when they became infected with BVDV. In the fall of 2014 a cohort of 26 PI calves were identified, all likely infected from the same source. The calves were purchased, euthanized, and a variety of fluids and tissues including serum, mesenteric lymph nodes and obex were collected. PCR amplification of the viral genome was followed by sequencing on an Illumina MiSeq. Genomes were assembled using MIRA against a reference strain and variant profiles were constructed at the nucleotide and amino acid levels. These profiles were compared to identify putative host- and tissue-specific mutations.

Results: Parts of the genome that are known to accommodate rapid changes (E2, NS2-3) also had a large variability in the studied PIs. Surprisingly, we found a substantial variability in certain regions of NS5b, a region generally considered as very conserved. Whether this variability is a characteristic of this specific virus strain or a more general finding in PI-derived BVDV using our analysis techniques warrants further investigation. Variant analysis shows hot-spots of variability in the E2, NS3, and NS5B regions of the genome. These areas tend to differ from the group mean variant profile more often than other genomic regions. There appears to be a stretch of very variable amino acid positions at position 1710-1750 of the amino acid sequence. These mutations include a variety of frame shifts and single amino acid changes. Although not previously documented, given that these mutations are occurring in the NS3 gene it is likely that they can contribute to biotypic conversion from noncytopathic to cytopathic strains and the subsequent induction of fatal mucosal disease.

Further work to understand the phenotypic importance of these observations is needed, but it is clear that BVDV exhibits a remarkable variability within and between PI animals.

Conclusions: A major driver of evolutionary change of viruses is the immune system, however, despite the absence of an acquired immune response against BVDV in these PI cattle, there are strong indications of viral variability that is of clinical and epidemiological importance. Our results demonstrates that the virus proteins can be modified in their amino acid structure leading to new strains and changes in their pathogenic properties. Understanding the extend and nature of these genetic changes will enable us to improve our responses through optimization of future vaccine development.

BVD

P03-003-026

Guidelines developed by the Australian Cattle Veterinarians organization for systematic management of BVDV in both beef and dairy production systems

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Objectives: The objective was to develop two stand alone beef and dairy BVDV management guides as resources to Australian Cattle Vet members to assist them in controlling and managing BVDV for their clients utilizing the tools available to them within Australia. The ultimate aim was to enable veterinarians to develop cost effective programs to define, manage, and monitor BVDV on their client's farms, accounting for Australia's varied farming practices.

Materials and Methods: With assistance from the Australian Cattle Veterinarians, a special interest group of the Australian Veterinary Association, a working group comprised of six veterinarians developed both a beef and dairy oriented management guide. The working group workshopped the guides over a series of face to face meetings, via teleconference, and via email. The first section of the guides related to the epidemiology and pathophysiology of the disease. The second section discussed the tools available to Australian veterinarians to manage the disease. The final section was designed to guide veterinarians by detailing testing options and control strategies. A series of flow charts were developed to accompany the advice.

Results: A complete beef and a complete dairy set of guidelines were each developed. The guides provide veterinarians tools to structure their advice to assist their client to understand the mechanisms by which BVDV is both transmitted and maintained on properties. The guide provided veterinarians a synopsis of the outcomes associated with exposure to the virus at varying stages of gestation, attempting to simplify their ability to relate the topic to their clients. The guide outlined the tools available to measure BVDV antibody levels, to identify PI animals, and to provide immunity via vaccination. Lastly, the guidelines provide step by step decision trees to assist veterinarians to cost effectively manage BVDV, creating a value proposition by utilizing immune status to discern the need to either vaccinate or screen groups for PI's.

Conclusions: The guides were produced by the Australian Cattle Veterinarians, a branch of the Australian Veterinary Association, as a tool for veterinarians working with cattle in Australia. The tools provided allow for cost effective management of BVDV relying on either vaccination or PI hunting dictated by serology. The strategies outlined allow for systematic herd level eradication and monitoring utilizing a value proposition rather than blanket approaches, making it far more palatable for Australian producers.

BVD

P03-003-027

Evaluation of 6 commercial ELISA kits for the detection of BVDV-specific antibodies in serum and milk using a large collection of field samples

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Objectives: Serological surveillance using antibody ELISAs is a commonly used tool to ascertain freedom of disease. Based on a previous study, 6 ELISA kits were selected for evaluation on a large number of serum and milk samples obtained in the field. The study goal was to evaluate performance characteristics of these kits and to verify

the observed lack of sensitivity of blocking ELISAs in serum and of milk testing, to evaluate whether pools of sera/individual milk samples can be used, and to assess whether it is possible to certify/monitor "BVD free herds" by means of serology on serum and/or (bulk) milk samples in the Belgian BVDV control programme.

Materials and Methods: 51 herds representative for the Belgian cattle sector (location, herd type, herd size, BVD status and vaccination status) were visited once. Serum samples were taken from all young stock (6-18 months) and from 20 randomly selected cows (mixed herds: 20 dairy and 20 beef cows). In dairy herds, one bulk milk sample and individual milk samples from the same cows which had been sampled for serum were taken. During the visit a questionnaire was filled out with the farmer to collect epidemiological information regarding BVD in their herd and vaccination, to be able to assign a BVD status to each herd: BVD free; BVD eradicated; BVD controlled; BVD infected less than 1 year; and BVD infected.

6 ELISAs were selected: 2/1 blocking and 2/1 indirect ELISA for serum/milk. Depending on the outcome of all ELISAs and using the virus neutralisation test (VNT) as a gold standard for serum, each serum and milk sample was assigned a positive, negative or discordant serological status. Next, all sera from unvaccinated herds were tested in VNT and VNT results were used as a reference for both serum and individual milk samples from the same animal. Using 2 by 2 tables we calculated true and false positive/negative results for each ELISA. These results were imported in STATA to calculate sensitivity (Se), specificity (Sp), Youden index, positive and negative predictive values, Kappa of Cohen and area under ROC curve.

Finally, weak/strong positive sera and individual milk samples were diluted in negative serum/milk to mimic pooled sera or bulk milk. Diluted samples were tested and analyzed.

Results: 3159 sera, 557 individual and 28 bulk milk samples were tested using 6 BVDV ELISAs and were assigned a positive, negative or discordant status. For serum samples, the percentage of positive samples was slightly higher for the indirect ELISAs than for the blocking ELISAs. For milk, the blocking ELISA performed better, though not significant, than the indirect ELISA, since the percentage of positively identified samples (73,2%) was similar to the percentage of the corresponding sera (75,9%), whereas the indirect ELISA seemed to be less sensitive (64,6%).

To assess the performance of each ELISA, only field samples (1308 sera and 293 individual milk samples) from unvaccinating herds (19) were used to exclude interference of vaccination. 3 out of 4 evaluated ELISAs on serum scored well for all parameters, whereas the anti-E0 blocking ELISA showed a lack of Se (93%), compensated by an increased Sp (99,1%). For milk, the blocking ELISA gain performed slightly better than the indirect ELISA both in terms of Se and Sp.

Pooling of serum and milk samples was investigated in each of the selected ELISAs since pooling of sera could reduce the costs of testing and to mimic bulk milk samples which are convenient and cheap samples to obtain. Our study showed that pooling should not be performed, since weak positive sera and individual milk samples were missed by all evaluated kits at small pool sizes. We also found that at this moment, a bulk milk sample does not reflect the BVDV status of a herd, since even in free or cleared herds a large proportion of cows are seropositive.

Conclusions: All kits performed well on individual serum samples, observing no difference between blocking or indirect ELISAs for samples from non-vaccinating herds. This led to the decision that both types of kits may be used for serological monitoring of BVDV free herds using individual serum samples in the Belgian BVDV eradication programme in the future. The decreased Se of the ELISAs using individual and pooled milk samples as a matrix and the finding that a bulk tank milk sample does not reflect the BVD status of a herd at the moment, indicates that it may be impossible to guarantee a "BVDV free" status of a herd based only on bulk milk testing.

BVD

P03-003-028

Nanowire-based electrochemical detection of anti-Bovine Viral Diarrhoea (BVD) antibodies in bovine serum

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Objectives: Rapid identification of BVD seroconverting individuals is essential in preventing costly outbreaks in naive herds. This is especially true in countries where BVD eradication is underway or complete. There is an unfulfilled need for new diagnostic techniques, suitable for on-farm analysis, that deliver early identification of virally exposed individuals, and yield the opportunity for immediate centralised data collection through remote devices. In this work we present an electrochemical on-chip fully integrated nanowire based immunosensor device for detection of BVD antibodies (Ab) in both buffer and serum in 20 minutes.

Materials and Methods: Gold nanowires electrodes, on-chip gold counter and platinum pseudo reference electrodes were fabricated on wafer-scale silicon substrates using hybrid e-beam/optical lithography, metal deposition and lift-off techniques. The on-chip nanowire electrodes were first modified with an electrodeposited polymer, using cyclic voltammetry. The carboxylic terminated polymer was activated using a mixture of EDC/NHS which allows for covalent immobilisation of the BVD capture molecules, i.e. BVD virus. The chip was then immersed in ethanolamine for 30 minutes to block the un-reacted active sites on the nanowire. Finally, the modified electrodes were exposed to known concentrations of Ab solution in phosphate buffered saline (PBS) for 20 minutes to confirm the suitability of the nanowires for label-free immunoassay applications. Each step of the assay was characterized electrochemically, illustrating a layer-by-layer build-up on the nanowire surface. The assay was repeated using serum samples again incubated for 20 min at room temperature. BVD virus and BVD monoclonal Abs specific to the envelop glycoprotein (Erns) of the virus were sourced commercially (APHA Scientific, UK).

Each step of the modifying process was electrochemically characterised using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). These experiments were performed in a 10 mM PBS solution containing 1 mM ferrocene monocarboxylic acid (FcCOOH) as a redox probe.

Results: The layer build-up from the functionalisation process, limits electron transfer of FcCOOH to the electrode. i.e. CV intensity decreased while the nyquist semicircles increased. Following immobilisation of BVD virus, the CV and nyquist spectra remained very similar to the ethanolamine deposition. This showed that the virus immobilised onto the majority of the nanowire surface. On exposure to the BVD Ab solution (PBS), Ab-Ag binding exhibited a strong decrease in the CV and a corresponding increase in the measured impedance. After successful demonstration of BVD antibodies detection in buffer, the BVD virus modified immunosensor was applied to detection of BVD antibody in bovine serum of known status (positive or negative). The same successful results were achieved after the modified wire was exposed to 1% positive serum incubated for 20 minutes. To confirm the bio-functionality and efficacy of the virus capture probe and to demonstrate the specificity of the sensor against BVD antibodies the sensor was exposed to BVD negative serum. We did not see any change in the electrochemical response, indicating that the sensor has the ability to discriminate between BVD seropositive and seronegative bovine samples. These sensors can be applied to the detection of the BVD virus using the same procedure, whereby the BVD antibody is immobilised onto the nanowire and the corresponding virus is detected.

Conclusions: This is the first electrochemical-based on-chip nanowire immunosensor device, using an electrodeposited polymer, to covalently immobilize BVD biomolecules. The nanowire-based BVD virus immunosensor allows specific detection of BVD antibodies in serum in 20 minutes. The ultimate aim is to reduce assay times thereby improve the on-farm applicability of this device.

BVD

P03-003-029

Monitoring of BVDV by testing antibodies to NS3 protein in calves vaccinated with an inactivated BVDV vaccine

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Objectives: On many BVDV free certified farms in the Netherlands, cattle are vaccinated with the inactivated vaccine Bovilis BVD (MSD Animal Health) to reduce the risk that new PIs are born. The BVDV free status of a farm can be monitored by 6-monthly spot blood testing of 5 calves in the age of 8 to 12 months for the presence of NS3-antibodies. Sometimes calves have been vaccinated before they have been tested. The aim of this study was to determine whether vaccination with Bovilis BVD might interfere with this monitoring system in calves.

Materials and Methods: In 8 BVDV free certified herds, 5 calves in the age group 8-12 months were vaccinated with Bovilis BVD according to manufactures instructions and 5 sentinel calves were left unvaccinated. Blood samples were taken from all calves on day 1 (first vaccination), day 28 (second vaccination), day 56, day 112 and day 168 and tested for NS3 antibodies in the Prionics BVDV ELISA. Blood samples taken on day 56, day 112 and day 168 were also tested for Virusneutralising antibodies (VNT). In one herd, blood samples from all calves between 1 and 16 months of age were tested for BVD virus.

Results: All vaccinated calves showed high VNT titers on days 56, 112 and 168. In 5 herds (A,B,C,D,E) neither vaccinated nor unvaccinated calves developed ns3 specific antibodies as measured by ELISA. In the herds F, G, H, all unvaccinated calves tested negative for antibodies as measured by ELISA. In 1 herd (F) 1 vaccinated calf showed a low titer in ELISA on day 56, but was tested negative at day 112. In 1 herd (G) 2 vaccinated calves showed low titers in ELISA on day 56, these titers were undetectable on day 112. In 1 herd (H) all 5 vaccinated calves showed moderate titers on day 56, these titers declined to very low titers on days 112 and 168. Because of the moderate titers on day 56, in this herd, blood samples from all calves between 1 and 16 months of age were tested for BVD virus. No PI animal was found.

In 6 out of the 8 herds included in this study vaccination of the calves did not at all interfere with the young stock monitoring using NS3- ELISA. In 1 herd, it was required to test an additional 5 calves by antibody ELISA to confirm the BVDV-free status. It remains unclear whether the relative high titers in the 8th herd were caused by i) the vaccination, ii) by transient BVDV infection, or iii) by infection from a PI that was already removed from the herd at the time of testing.

Conclusions: Young stock monitoring can be applied in herds vaccinated with the inactivated BVDV vaccine used in this study. All vaccinated calves developed high neutralizing antibody titers. Only a small proportion of vaccinated animals developed low, short lasting titers

of BVDV-NS3 specific antibodies as measured by NS3-ELISA, potentially hampering young stock monitoring. It is therefore recommended to either take blood samples before vaccinating or to monitor on vaccinating farms by antigen detection methods.

BVD

P03-003-030

Application Of An Indirect Elisa For The Discrimination Of Bvdv 1, 2 And 3 (Hobi-Like) Infection, Based On Anti-E2 Antibodies In Bulk Milk

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Objectives: BVDV is a member of genus Pestivirus in the family of Flaviviridae. In addition to the recognized species, 4 additional Pestivirus species have been proposed but remain officially unrecognized. One of these new putative species is BVDV type 3 (Hobi-like). BVDV structural protein E2 is the major target for neutralizing antibodies and represents the only target antigen for discrimination between different BVDV species based on cross-neutralization assay. In this study the E2 proteins of BVDV1, 2 and 3 were produced as secreted recombinant and employed for the development of a triple diagnostic ELISA on bulk milk samples.

Materials and Methods: To isolate the full-length BVDV1, 2 and 3 E2 genes, RNA was extracted from Madin-Darby bovine kidney (MDBK) cell cultures infected with the three field strains of BVDV using a EZ1@ Virus Mini kit V2.0 (Qiagen, Hilden, Germany) and the BioRobot EZ1 extractor. It was selected a variable region of the three sequences, in order to differentiate the three circulating virus types. Gene fragments ligated with pSecTag2/Hygro plasmid (Invitrogen, USA) allowing an efficient intracellular sorting and secretion in the medium of transiently transfected mammalian cells. Sub-confluent human embryonic kidney (HEK293T) cells were transfected according to standard protocol. Sheep were immunized with inactivated native virus preparations of BVDV 1, 2 and 3 and serum titers were normalized according to homologous neutralization assay. Checkboard titration of the recombinant antigens was carried out using normalized control sera for maximum discrimination. In ELISA protocol, milk samples were used undiluted and incubated for 120' while control serum samples were diluted 1/20 in PBS 1.25% casein. Milk samples were collected from 286 dairy farms located in the territory of Piedmont Province of Cuneo. Bulk milk of each farm was collected in pool including not more than 40 lactating animals, for a total of 393 milk pool samples. During sampling, BVDV status and vaccination program was unknown for all the dairy farms.

Results: Sequence analysis of each construct showed the correct in frame cloning of the three sequences. E2 protein expression resulted different between three types: E2 of BVDV1 resulted two logs more expressed than BVDV3. Antigen production was confirmed using sera from sheep immunized against the three viral species, diluted and used as positive controls according to neutralization test. Preliminary data underline, as expected, the presence of BVDV1 in majority of dairy herds, confirming the wide use of monospecific vaccination and BVDV1 circulation. However, in few occasions, reactivity against BVDV2 and BVDV3 was detected. Interestingly, in the latter herds, pools from the same herd reacted in a similar manner, supporting the specificity of the

ELISA signal. Further investigation in one BVDV2 positive herd allowed identification of several PI animals infected with BVDV-2a, as detected by sequencing of RT-PCR amplified UTR region and representing the first report of BVDV2 circulation identified in North West Italy. The low antibody titre against BVDV3 recorded in lactating animals older than 3 years in a second farm suggested a viral extinction as no PI animals were detected.

Conclusions: The results of the present study confirm that BVDV1 seroprevalence is high in Piedmont as it is along Italian territory, confirming both monospecific vaccination and BVDV1 circulation. However, for the first time, the circulation of unusual BVDV strains have been predicted using serological test, as in the case of an active outbreak of BVDV2 which have been recognised in Italy a couple of times. This new assay therefore represents an useful tool for large scale monitoring of unusual and atypical pestivirus, leading to assess herd immune status or active circulation versus three BVDV species.

BVD

P03-003-031

Risk of pestivirus contamination of bioproducts used for immunoprophylaxis and diagnostic purposes

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Objectives: The contamination of fetal bovine serum (FBS) with pestivirus, especially Bovine Viral Diarrhea Virus (BVDV) is a common problem of concern for the safety of vaccine, plasma-derived bio-products, which may also influence the results of cell culture-based diagnostic testing. In the recent years, the new pestivirus species including BVDV-2 and Hobi-like virus emerged in Polish herds. The main objective of the study was the assessment of the risk of pestiviral transmission by defining the levels of contamination of vaccines with pestiviruses which are used in cattle management and immunoprophylaxis, as well as FBS for diagnostic use.

Materials and Methods: A total of 179 vaccines against both viral and bacterial disease for use in various animal species (68 were live attenuated or marker vaccines) and 22 commercial sera originating mostly from South America and Australia including fetal bovine serum, fetal equine serum, horse serum, newborn bovine serum and newborns calf serum and were tested for the presence of BVDV and related pestiviruses. Viral RNA was extracted using QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). All samples were tested for the presence of BVDV viral genome by RT-PCR and real-time RT-PCR. Pan-pestivirus primers flanking 5'UTR region of viral genome were used for standard RT-PCR, whereas in real-time RT-PCR, primers and probes specific for BVDV-1, BVDV-2 and atypical bovine pestivirus (HoBi-like) which allow species differentiation were applied. The presence of BVDV antigen was tested with commercial E^{ms} BVDV Ag/Serum Plus ELISA kit (IDEXX, Liebefeld-Bern, Switzerland). Additionally the serum samples were tested for the presence of BVDV specific antibodies using a commercial BVDV Total Ab ELISA kit (IDEXX).

Results: Contamination of pestivirus was detected in 6 (3.3%) of tested vaccines. Four vaccines were positive in standard RT-PCR and additionally BVDV antigen was detected in another two vaccines by ELISA. Only one vaccine was positive in BVDV-1-specific real-time RT-PCR while BVDV-2 and HoBi-like pestiviruses were not identified as contaminants of the vaccines. In the case of vaccines against pestivirus (BVDV, CSFV) infections, 20 out of 21 of such vaccines gave positive

result in antigen ELISA. The only antigen ELISA negative vaccine gave positive result only in real-time RT-PCR. Additionally, 7 of pestivirus vaccines were confirmed in RT-PCR and 13 in real-time RT-PCR.

Seventeen batches (31.8%) of commercial sera were found to contain at least one species of bovine pestivirus, but only twelve of those were positive in standard RT-PCR. In real-time RT-PCR, 11 batches were found positive for BVDV-1, 3 batches positive for BVDV-2, and 9 batches positive for the HoBi-like pestivirus. BVDV antigen was found only in three samples from two suppliers using antigen ELISA. None of the foetal equine serum and horse serum was antibody positive or contained viral genetic material. Specific pestivirus antibodies were detected in 4 (18.2%) commercial sera.

Conclusions: The contamination with BVDV-1, BVDV-2 or HoBi-like pestiviruses of bioproducts especially commercial FBS available at the local market and used in immunoprophylaxis and diagnostics in Poland, remains a threat for pestivirus transmission as well as may falsify the results of diagnostic test based on FBS usage. While, the risk of pestivirus infection through a vaccine seems low, the use of the vaccines contaminated with BVDV antigen may provoke seroconversion which may result in failure of BVDV eradication program.

BVD

P03-003-032

Genetic Diversity of Bovine Viral Diarrhea Virus and Frequency of Genotypes and Subtypes in Galicia (NW Spain)

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Objectives - to investigate the frequency and diversity of BVDV circulating in the Galicia region (NW Spain), during the period 2013-2016, in order to support the design of optimized control programs.

Materials and methods - 147 BVDV strains were typed from 145 cattle and 2 sheep. For analysis RNA was isolated from samples using the QIAamp Viral RNA Mini Kit (Qiagen, Manchester, UK). cDNA was synthesized from template RNA using the AffinityScript Multiple Temperature cDNA Synthesis kit (Agilent Technologies, CA, US) according to the manufacturer's instructions. Then a 288-bp DNA product from the 5'UTR region was PCR-amplified using primers 324 and 326 as previously described. Amplified DNA fragments were purified by ExoSAP-IT treatment (USB Corporation, OH, USA) and sequenced at the Sequencing and Fragment Analysis Unit of Santiago de Compostela Sequences were converted to FASTA format using Chromas Lite 2.1.1 and imported into MEGA 6. Phylogenetic trees were constructed using the neighbor-joining method and validated using bootstrap analysis with 1000 replicates. Evolutionary distances were estimated using the Kimura two-parameter method.

Results- 147 BVDV strains were typed, of which 144 (97.9%) belonged to species BVDV-1 and 3 (2.1%) to BVDV-2. Of the 147 strains, 125 (85%) were typed as BVDV-1b based on 5'UTR analysis and comparison with reference strains. The virus in other 3 samples was identified as BVDV-1e, while the viruses in 9 other isolates were most closely related to BVDV-1d and in 3 to 1p. One isolate each was assigned to groups 1a, 1h, 1k and 1l. One of the BVDV-2 isolates was classified as 2a; it came from a PI cow that was 31 months old when diagnosed and that had already calved once. Analysis of the calf (male) was impossible because he was slaughtered soon after birth. Thrombocytopenia, hemorrhage or other clinical signs attributed to highly virulent strains of BVDV-2 were not observed. The other two BVDV-2 isolates came from a sheep farm in which reproductive problems (mainly abortions) were observed. In this farm, after negative result to the Border disease virus test, BVDV was isolated, which was classified as 2d.

Conclusion - This report, the first study of BVDV genetic diversity in Galicia, indicates that BVDV-1b is the predominant group, as in other European countries. This report also identifies several BVDV types not yet described anywhere in Spain, and it shows for the first time that BVDV-2 is present in Galicia. To our knowledge, this is the largest BVDV typing study ever reported for Spain. Our results identified ten different BVDV subgroups in Galicia.

BVD

P03-003-033

Noncytopathic bovine viral diarrhea virus 2 inhibits the expression of major histocompatibility complex II molecule DQ, not DR

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Objectives: Bovine viral diarrhea virus (BVDV) is an economically important viral pathogen that can affect on the productivity of cattle and lead to substantial economic loss on the livestock industry worldwide. BVDV infection is associated with both acute and persistent infections depending on epidemiological circumstances. BVDV exists in two biotypes, cytopathic (cp) and noncytopathic (ncp) based on their effect on cultured cells.

Materials and Methods: Blood samples were collected, bovine PBMC were isolated using Histopaque gradients, and resuspended in RPMI 1640 medium. To separate monocytes, non-adherent cells were removed, and adherent cells were washed with sterile PBS, detached from the plates, and pelleted by centrifugation at 650 g for 10 min. For virus infection, 5 × 10⁶ monocytes were added to a 6 well plate and infected with cp and ncp BVDV biotypes at the same MOI of 0.002 for 48 h. After 48 h, ncp BVDV1, ncp BVDV2, and mock-infected cells were harvested. The expression of major histocompatibility complex class

(MHC) II molecules DR and DQ in each BVDV-infected monocytes was measured by western blot, flow cytometry and real-time RT-PCR.

Results: The results showed that ncp BVDV1-infected monocytes displayed the expression of total MHC class II on their surface by flow cytometry, which showed a similar expression of mock-infected cells. In contrast, there was significant down-regulation in ncp BVDV2-infected cells. However, the expression of DR in ncp BVDV2-infected cells was unchanged compared with DQ expression. The expression of DQ by real-time RT-PCR was markedly reduced in ncp BVDV2-infected cells. A significant reduction in the levels of DQ protein was observed in ncp BVDV2-infected monocytes compared with ncp BVDV1-infected cells. Taken together, these results indicate that the down-regulation of MHC class II in ncp BVDV2-infected monocytes is due only to the reduction of DQ, but not DR.

Conclusions: Our results suggest that ncp BVDV2 inhibits antigen presentation to immunocompetent T cells compared to ncp BVDV1 infection. The present results suggest that ncp BVDV2 fails in performing viral control and clearance as well as has detrimental effects in the animals, which results in defective antigen presentation, enhanced virus dissemination, and severe immunosuppression. Our results thus provide insight to better comprehend the pathogenesis of ncp BVDV2 infection.

BVD

P03-003-034

BVD testing strategies in neonatal calves: serial application of ELISA and real time RTPCR and evaluation of the potential “diagnostic gap”

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Objectives: All Irish calves are tested for BVDV as part of a national eradication programme. After an initial positive or inconclusive result, a confirmatory test on a blood sample may be used to distinguish persistent from transient infections. This testing must take into account the potential for false negative results due to maternally derived antibodies (diagnostic gap) when using ELISAs. However, individual rRTPCR testing is costly. Therefore samples are screened by ELISA, with negative samples further tested by rRTPCR. The objective of this study was to investigate the performance of both tests with particular emphasis on the diagnostic gap.

Materials and Methods: The national eradication programme permits animals with a positive or inconclusive result on an initial tissue tag test to be subjected to a confirmatory test to determine if it is persistently or transiently infected with BVDV. A commercial BVD antigen capture (Ag) ELISA (IDEXX) was performed according to the manufacturer's instructions on 6,497 serum samples submitted for this purpose to the Central Veterinary Research Laboratory in 2013. RNA was also extracted from these samples using an automated extraction robot and tested for BVDV by real time RTPCR. Samples with cycle threshold (Ct) values below a pre-set cut-off limit were considered positive. A BVD Virus Neutralisation Test (VNT) in a microtitre format was performed in serial twofold dilutions up to 1/4096 on a subset of heat-inactivated serum samples.

Results: Of the 6,947 calf samples tested, 1,921 (28%) were found to be negative on Ag ELISA and were retested using BVD rt-PCR. 285[DG1] of these proved ELISA negative/PCR positive (ENPP) (indicating a

diagnostic gap in 4% of samples). Both the age at blood sampling and the interval between initial ear notch and confirmatory blood sampling were significantly lower in this ENPP group at 46.1 and 35.7 days respectively, compared calves whose confirmatory blood samples tested positive by ELISA (62.0 and 49.1 days respectively). The rPCR results for this ENPP group showed a range of CT values, with 17% having Ct>34 and 4% having Ct<25. The serology results for this group showed a range of titres. While 44% of samples had high titres (>1/4096), 5% of samples had titres < 1/8.

Conclusions: The ELISA is a robust assay well suited to disease eradication programmes, but has a lower analytical sensitivity than rRTPCR and is susceptible to interference from BVD-specific antibodies in serum. Reliance on ELISA testing alone would result in 4% false negatives, highlighting the importance of further testing of ELISA-negative samples by rRTPCR to individual farms and control programmes alike. Based on this study, negative ELISA results on serum samples are only accepted within the Irish eradication programme when they come from calves aged over 75 days.

BVD

P03-003-035

Detection Of Infectious Bovine Rhinotracheitis, Bovine Viral Diarrhea And Bovine Respiratory Syncytial Viruses In Nasal Exudate From Calves By RT-PCR

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Objectives: This study shows the results obtained from 5 years samplings with a diagnostic tool (BOVIRESP®) based on Real Time (RT-PCR) analysis to determine the presence or absence of Infectious Bovine Rhinotracheitis virus (IBRV), Bovine Viral Diarrhea virus (BVDV) and Bovine Respiratory Syncytial Virus (BRSV) in nasal swabs from calves suffering acute outbreaks of respiratory disease in Spanish feedlots.

Materials and Methods: A total of 1.614 nasal swabs have been analyzed from pneumonic calves from 2009 to 2013. Nasal exudate from 6 different calves from one feedlot with suspicion of virus shedding were collected using the diagnostic kit. Refrigerated samples were sent to DIAGNOS (HIPRA, Spain). Once in the laboratory, automated extraction of viral DNA and RNA (QIAmp DNA and RNeasy minikit, Qiagen) was realized (QIAcube, Qiagen), then specific amplification of IBRV, BRSV and BVDV genetic material was realized through three different RT-PCRs adapted from previously described protocols. A final report with RT-PCR results was generated for each specific farm. Data generated in reports from 2009 to 2013 was analyzed.

Results: A total of 269 reports were delivered. One hundred and five of these reports (39%) were positive to at least one of the analyzed pathogens. Fifty-four (51.4%) of these reports were RT-PCR positive to BVDV, 50 (47%) were positive to BRSV and 14 (13%) were positive to IBRV. Thirty reports (12.3%) were positive to more than one analyzed virus. In 48% of BRSV-positive reports, samples had been collected between September and December (autumn in Europe). Samples from IBRV and BVDV positive reports had been collected along the year without observable differences. In contrast with the clinical perception of many veterinarians, it is noteworthy that the most common isolated pathogen is BVDV. This fact suggests a potential major participation of this virus in the Bovine Respiratory Disease (BRD) etiology, probably as an immunosuppressor. The seasonality of the detection of positive animals to BRSV is remarkable, being greater in autumn.

Conclusions: This diagnostic kit (BOVIRESP®) based on RT-PCR is currently well established as an important tool of diagnostic support for the veterinary practitioner. The results obtained suggest the necessity of studying more deeply the pathogenic role of BVDV in BRD, as well as considering BRSv as a frequent viral pathogen in BRD.

BVD

P03-003-036

Evaluation of the presence of antibodies to BVDV I and II in cattle on a farm with rigorous control on the transit of animals

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Objectives: BVDV is a worldwide spread disease in most countries there is a high rate of seropositive animals above the age of three years, ranging between 60 and 90%. The great antigenic diversity observed among isolated strains of BVDV has special meaning for epidemiology, diagnosis, immunization strategies and control of the disease. The objective was to verify the presence of antibodies and cross-neutralization against BVDV - I and BVDV - II. Also assess whether the fact of not having traffic farm animals helps control BVD.

Materials and Methods: This study used 114 female cattle, aged 02-84 months herd for milk and unvaccinated for BVDV, coming from herds located in the city of São Carlos, São Paulo, Brazil. It is a closed herd, meaning there is not transit animals. Two harvests were performed, the first Jan / 2015 and the second Feb / 2015, ie, one month apart. The serodiagnostic was conducted by the VN method, as described by OIE, using the BVDV-I NADL and BVDV-II strains VS253. The test cutoff point was 10. The samples were serially diluted in log base 2, from the 1:10 dilution to 1: 5120. The property has a history of abortion, of the 114 evaluated animals, 14 animals showed abortion along its life.

Results: The animals studied only 7% (8/114) were positive for BVDV -I, these five were reactive to both the strains used. The analysis with BVDV -II revealed that 5% (6/114) animals had antibodies against this strain . The antibody titers ranged from 10 to 320 for BVDV -I, the titers were lower when compared to BVDV -II, the highest titer BVDV-I was 80 and higher for BVDV-II was 320. Surprisingly the amount of positive animals is much lower than expected. None of the 14 animals that have aborted any time during their life presented titer of antibodies against BVDV-I and BVDV-II. Surprisingly the amount of positive animals is much lower than expected. None of the 14 animals that have aborted any time during their life presented titer of antibodies against BVDV-I and BVDV-II.

Conclusions: It is concluded that there was a low frequency of occurrence of bovine reagents to BVDV. It was demonstrated that to use of more than one viral strain, increased the sensitivity of detection of seropositive animals in the VN. The farm maintains rigid control of the animals, and the low percentage of positive animals shows that the handling and care of infectious diseases are valuable tools now to maintain low levels of BVD. Abortions property can not be linked infectious causes, since none of the animals with a history of abortions had title virus detection.

BVD

P03-003-037

Quantifying The Risk Of Spread Of Bovine Viral Diarrhoea Virus (Bvdv) Between Contiguous Herds In Ireland

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Objectives: The control of bovine viral diarrhoea virus (BVDV) mainly focuses on the identification and restriction of persistently infected (PI) animals. However, other transmission pathways can also result in new breakdowns, including the movement of animals pregnant with PI calves and the spread of infection between contiguous farms. Contiguous spread is likely an important problem in the BVD eradication programme in Ireland, given the spatial distribution of residual infection, and the highly fragmented nature of land holdings on many Irish farms. The objective of this study was to quantify the risk of BVD spread between contiguous herds in Ireland.

Materials and Methods: Multivariable logistic regression models were used to estimate the infection risk of a herd having BVD positive calves in January to June 2014 (the study period) when contiguous to a herd that had at least one BVD positive calf born in 2013. The models included risk factors relating to the study herd and to neighbouring herds. Separate multivariable models were built for each of four "PI-neighbour" factors relating to the presence of BVD+ animals and/or the presence of offspring of PI breeding animals.

Results: In total, 58,483 study herds were enrolled. The final model contained the province, the log of the number of calf births registered during the study period, the number of cattle purchased between January 2013 and January 2014, and with a two-way interaction between the number of animals of unknown BVD status in the study herd and the PI-neighbour risk factor. When the number of PI-neighbour herds was used as the PI-neighbour risk factor, the odds ratio (OR) associated with the number of PI-neighbour herds ranged from 1.07 to 3.02, depending on the number of unknown animals present. To further explore the risk associated with PI-neighbour factors, the models were repeated using a subset of the study herds (n = 7,440) that contained no animals of unknown status. The best fitting model including "any PI-neighbour" as the PI-neighbour factor and also contained the log of the number of calf births registered during the study period and the number of cattle purchased. The OR associated with "any PI-neighbour" was 1.92 (95% C.I. 1.37-2.70).

Conclusions: This study provides the first quantitative information on the risks posed by the presence of BVD+ animals in neighbouring herds and also highlights the importance of clarifying the BVD status of animals that have not yet been tested in the context of the Irish eradication programme.

BVD

P03-003-038

Lessons learnt from a cross-sectional field survey: how to implement serological monitoring of BVD-free herds in the Belgian BVDV eradication program?

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Objectives: In order to provide recommendations regarding serological surveillance strategies to certify and/ or monitor freedom of infection in Belgian herds using BVDV-specific antibody ELISAs, 51 Belgian herds were sampled and serological results were analysed taking into consideration epidemiological parameters at the individual and herd level. The epidemiological data was collected through a questionnaire filled out in each of the visited farms the day of sampling. Factors with relation to age of sampled animals, sample matrix, type of ELISA, number of animals to sample, design prevalence and vaccination status were evaluated.

Materials and Methods: 51 herds representative for the Belgian cattle sector were visited once. Serum samples were taken from all young stock (6-18 months) and from 20 randomly selected cows (mixed herds: 20 dairy and 20 beef cows). In dairy herds, one bulk milk sample and individual milk samples from the same cows sampled for serum were taken. All samples were tested for BVDV-specific antibodies using different ELISAs and a positive, negative or discordant final serological status was assigned to each sample. An animal-level database was created in Excel® for all individual samples with other variables (ID, age, sex, herd type,...) extracted from the national livestock databank (SANITEL).

During the visit a questionnaire was filled out to characterize each herd and to collect epidemiological information regarding BVD status, control measures in place, vaccination practices, risk factors for BVD introduction, detection of PI animals in the herd,... Answers were recorded in a herd-level Access® database. Then, a BVD status was assigned to each herd: BVD free; BVD eradicated; BVD controlled; BVD infected less than 1 year; and BVD infected.

Using SAS 9.2.® software, the 2 databases were merged taking the herd ID as matching variable. Descriptive statistics were performed to identify factors that influenced the serological status of samples and herds. Subsequently these factors were included as covariates in a univariate logistic regression using the serological status of the sample as dependent (response) variable. Significant covariates were kept and put in a final multivariable logistic model.

Results: Among several explanatory variables, herd production type, participation to auctions, vaccination status against BVD, age of the animals as well as BVD status of the herd clearly influenced the obtained serological results. After considering the seroprevalence observed in function of age class, vaccination and BVD herd status, the following recommendations were given to the stakeholders with relation to serological monitoring of BVD-free herds:

- serological monitoring should preferably be performed in animals at the age of 6 up to 18 months.
- only unvaccinated animals should be sampled for serological monitoring.
- bulk milk testing cannot be used at this stage to monitor the possible exposure of the herd to BVDV infection.
- a case detection protocol is advised for annual serological monitoring

of BVD-free herds, designed to detect a minimum prevalence of 15%, with a higher seroprevalence indicating a possible recent circulation of BVDV in the herd.

- annual monitoring of BVD-free herds can be performed by testing 15 to 19 young unvaccinated animals in function of herd size. All tested animals should be seronegative.
- Alternative strategies to monitor BVD-free herds: continuous testing for BVDV of all new-born calves, annual Ag testing of all animals which have not yet been certified non-PI, serological annual testing of larger number of animals to evaluate the herd seroprevalence with an acceptable precision,... The choice of the monitoring system should be adapted to each herd and should take into account practicability and financial cost in function of the herd size.

Conclusions: This cross-sectional serological survey coupled with an epidemiological survey delivered very useful data and information to anticipate the serological monitoring of BVD-free herds in the Belgian BVDV eradication programme initiated in January 2015. It has been shown that serological monitoring can be used as a tool to maintain a BVD-free herd status provided that it is adapted to the epidemiological field situation and in particular takes into consideration age of the animals, vaccination status and herd seroprevalence observed in function of exposure to BVDV.

BVD

P03-003-039

Assessing testing of serum pools as a cost-effective method for establishing the bovine viral diarrhoea (BVD) status of a herd

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Objectives: Bovine viral diarrhoea (BVD), caused by infection with bovine viral diarrhoea virus (BVDV), is one of the most economically important endemic diseases of cattle. Within disease control programmes, serology is often used as a rapid and cost-effective intermediate step to identify herds that are likely to contain persistently infected (PI) animals before proceeding to whole herd testing. The objective of the present study was to evaluate the use of serum pools as an alternative to the testing of individual samples for the detection of antibodies to bovine viral diarrhoea virus (BVDV) using a range of enzyme-linked immunosorbent assay (ELISA).

Materials and Methods: Between 5 and 10 serum samples from young stock (12-14 months) from 300 herds participating in a BVD seroprevalence study in Northern Ireland and representing a range of 'within herd [sample] prevalence were initially tested for antibodies to BVDV using a p80 ELISA kit (LSI Vet BVD/BDV p80 blocking one step kit, Life Technologies). Of these, 135 were vaccinating the herd for BVD with an inactivated vaccine and 165 were not. Thereafter serum pools consisting of an aliquot from each individual sample tested per herd were prepared and tested with a range of p80 and whole virus antibody ELISA kits and protocols. Results obtained were compared to the proportion of seropositive animals in each pool as tested with the LSI p80 kit using the individual sample protocol. The characteristics of the tests used were evaluated using WinEpiscope 2.0 (<http://www.clive.ed.ac.uk>).

Results: When testing the whole set of herds, all kits except one of the

protocols described for the LSI kit (LSI epidemiological protocol- LSI E) obtained a relative sensitivity of pooled sera relative to individual animal results of >94% (94-99.9%). All p80 kits returned a relative specificity (Sp) >87% (87-100%). The whole virus kits had a lower specificity (between 73 and 77%). Kappa values were over 0.7 for all kits with the exception of the LSI E. Three out of the five p80 protocols received a higher kappa value (>0.8) than the whole virus kits (>0.69). The other two p80 kits obtained kappa values of 0.7 and 0.35. When only non-vaccinated herds (n=165) were included in the analysis, the specificity of all the kits achieved 100%. The sensitivities however decreased slightly (between 0.5% -2.2%). Kappa values improved for all whole virus protocols and for most of the p80 kits.

Conclusions: The present study indicates that testing of serum pools for antibodies to BVD can provide a cost effective method to assess the status of a herd. Herds vaccinating with an inactivated vaccine should be tested with p80 kits in order to minimize false positive results. In herds vaccinated with a modified live vaccine, vaccine-induced antibodies will be detectable with both kits so only unvaccinated animals should be sampled. Test performance could be improved further by optimising the cut off but it should be chosen in light of the objective of the testing and with the understanding that it will have an effect in the Se and Sp achieved.

BVD

P03-003-040

Reconstructing BVDV infection in Northern-Belgium using network analysis

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Objectives: A main influencer of the spread of BVD (Bovine Viral Diarrhoea) is the trade of persistently infected (PI)-cattle. Starting from the 1 of January 2015, Belgium takes up a national BVD-control programme though systematic detection of PI cattle and banning them from trade. This abstract reconstructs the trade of (known) detected PI-animals in the voluntary phase of BVD-control (2010-2014) in Northern-Belgium through network analysis.

Materials and Methods: For a five-year period (1 Jan 2010– 31 Dec 2014) before the national BVD-control programme, all BVD-antigen test results with complete animal ID were collected. All cattle tested positive for BVD-antigen at Animal Health Care Flanders & not (re)tested negative were assumed as PI-animals and used in this analysis. Using I&R data the life-trajectory of (known) detected PI-animals with livestock movements was reconstructed. Network analysis was performed using NodeXL© and geolocation though Gephi© & Google Earth©.

Results: A directed graph or 'BVD PI-network' of 3,052 vertices (cattle farms) and 4,011 Edges (PI-transports) was constructed. Graph accounts for (known) detected PI-movements between 3,052 cattle and veal calf herds . The actual trade of PI-animals is underestimated since Not all PI animals were detected (e.g. only approx. 10% of annual traded animals is tested at purchase). The actual dispersion of BVD is underestimated since other transmission routes are not graphed (e.g. transiently infected animals, Trojan pregnant cows, indirect transmission, etc.).

Conclusions: There was evidence of (temporarily) presence of PI-animals in 16.7% of cattle herds in Northern-Belgium. This abstracts clearly illustrates the need for a systemic control of BVD in Belgium. This

abstracts was used to help decision makers concerning the start of a national control Belgium in Belgium in 2015.

BVD

P03-003-041

Case report: Severe loss on a Belgian dairy farm presumably caused by an infection of Bovine viral diarrhea virus type 2 a

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Objectives: This abstract describes a clinical case of Bovine Viral Diarrhea (BVD) on a dairy farm in the eastern part of Belgium. It all started in April 2012. After the first severe clinical symptoms the situation was monitored until BVD apparently disappeared from the farm in spring of 2015. BVDV is divided in two subtypes, BVD type 1 and BVD type 2. In the US and Canada 50 % of BVD infections are caused by BVDV type 2. In Europe this is until now approximately maximum 5 %. In this case contact with BVDV type 2 has been confirmed.

Materials and Methods: It concerns a dairy farm with approximately 179 animals present, of which 100 lactating cows. The farm is well managed and uses the zero grazing principle. April 12, 2012 the veterinarian was called in because several dairy cows became ill and an overall production drop of one third was noticed. Symptoms were high fever (>40° C), severe watery diarrhea sometimes with a hemorrhagic aspect, fast dehydration and collapse in a short period of time. Within a fortnight 17 adult cows died. Some cows in the herd developed fever but recovered. BVD was already monitored on this farm by means of a yearly spot test. I.e. Sample animals of 6 to 13 months of age for BVD antibodies to monitor virus contact. On March 29, 2012: 14 days before the massive clinical outbreak, a spot test was performed and results were completely negative. BVDV infection was suspected but could not be confirmed. Several cows were tested and tests were negative. A bulk milk test BVD Ag PCR was negative at the time of disease. Several other analyses were performed (Salmonellosis, Ehrlichiosis) but all came back negative. May 12, 2012 was the first time contact with BVDV could be confirmed, three cows were found positive for BVD antibodies. As of June 27, 2012 every newborn calf was tested by ear notch sampling with a BVD Ag ELISA test. Several calves were negative but October 4, 2012 the first positive calf (PI) was born. The moment this calf was infected fits with the period of the severe clinical outbreak. Several calves were born positive during the next years, even after taking the best possible precautions.

Results: August 27· 2014 the veterinarian was asked to examine two sick animals. These two heifers 21 months of age were found positive BVDV type 2 but both tested negative at birth by BVD Ag ELISA on an ear notch sample. One died acutely and probably suffered an acute transient infection. The other one was always a poor doer. This animal was euthanized and by examination of lymph nodes, organs and ear tissue it could be confirmed as a PI animal. Because of the level of virus (Cycle time values PCR) found in every organ and tissue. The virus was identified as BVDV type 2 a. The respective mothers of these animals tested negative but both animals showed antibodies with a more elevated level against BVDV type 2. September 30, 2014 the whole herd was sampled and tested for BVD Ag. No PI animal was identified. After that on March 6, [†] 2015 again a PI was born and the infecting virus was identified as the same BVDV type 2 a. In January 2015 vaccination was started. Another calf, last one in row, was born positive on June

4, 2015. This calf must have been infected at a time were no other PI animal was present on the farm, because on September 30 2014 all herd was screened. The mother of this calf was bred in September the only possible time of infection to develop a PI could take place between October 2015 and January 2016. This infection had to be caused by transient infected animals on the farm.

Conclusions: The origin of the first disease outbreak in the dairy cows could not be identified. By deduction the virus BVD type 2 must have entered the farm in March 2012. The diseased heifers in august 2014 were tested negative at birth by ear notch BVD Ag ELISA. It is very likely that at least one of these test results was false negative. This case report shows that to eradicate BVD more is needed then only tracking PI's and eliminating them. Diagnostic testing is coming too late when it concerns BVD. In addition to monitoring new entrance of the BVD virus it is necessary to elevate bio security measures and to protect the animals by vaccination.

Comments: The author was veterinary practitioner in a cattle practice for 20 years. Then he worked for three years at an Animal health service organization in Belgium. Since April 14, 2014 he joined Boehringer-Ingelheim as Field Technical Manager Cattle & Horse.

BVD

P03-003-042

BVD control on dairy farms: a in clinic approach to engage farmers

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Objectives: Bovine viral diarrhea (BVD) is an important disease and causes health and welfare issues and economic losses. Farmers want healthy cows and beneficial financial results without problems and they consider animal welfare a very important issue nowadays. Therefore they want no BVD on their farm.

The farmer-clients of this clinic are well aware of the effect of BVD and most farmers participate in a BVD control program. This is a case report of an in clinic approach how awareness was raised, to stimulate farmers to control BVD and to stay engaged.

Materials and Methods: The intrinsic motivation of the veterinarians is important. Our practice has SMART targets on BVD. For each vet records of the number of farms working on BVD control are present. BVD vaccination is part of our control program. Team agreements on the following were part of the strategy; speak with one voice: all vets have the same approach, up to date and adequate knowledge of BVD and control programs, use the AIDA model to convince and motivate farmers. AIDA model is a stepwise procedure:

Attention Bring BVD to the farmers attention. The method is depending on the type of farmer. Examples are written articles, lectures and study groups. The strategy is to keep the messages simple and uncomplicated; "BVD eradication and control is simple!"

Interest The next step: how to get the farmer really interested in BVD control. What are the losses caused by BVD on the farm? BVD control is financially beneficial. What does it bring the farmer? Repetition of easy messaging is key.

Desire Often stimulated by external factors, such as experiences of other farmers, a herd health plan or stimulated by the dairy industry.

The farmer is interested to learn more. A clear and easy approach will get him ready for the next step.

Action The farmer is ready to control BVD and willing to participate. In this part it is important that all vets have the same approach. Is the farmer convinced to start vaccinating?

Every vet or person has their own specialty depending on his or her character. Working in a team has an advantage because for every step in the AIDA model one can consult a more convincing vet.

Results: The number of farms participating in a BVD eradication and control program (including vaccination) increased in the last 3 years from 69% of the farms to 83% in 2015. The variation in the participation rate of farmers varies per vet from 74% to 100%; likely due to "inter-veterinarian" difference in competences and communication skills. The number of vaccinating BVDV free herds went up from 5% to 10% of total farms.

The motivation of the vets and farmers to control BVD has increased. By following the AIDA model and the approach as a team BVD control in this practice is considered successful.

Conclusions: BVD control should be in the hands of the vets because of their knowledge and expertise on the disease. Awareness within the team and a uniform approach of BVD control will stimulate and motivate veterinarians. This case description shows the success of this approach by the increased number of farms participating in a BVD control program and BVD vaccination.

BVD

P03-003-043

First results of the bovine viral diarrhea control plan in Southern Belgium

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Objectives: Since first of January 2015, a compulsory control plan against the bovine viral diarrhea virus (BVDV) is implemented in Belgium. This program, like in German and Luxembourg, is based on two cornerstones:

- (1) a systematic screening of the calves at birth, and of the dams giving a positive calf;
- (2) a marketing ban (except for slaughter) and an interdiction of pasture for the detected or suspected PI animals.

This study provides a descriptive analysis of the results obtained in Southern Belgium during the first nine months of the control plan.

Materials and Methods: Based on the informations retrieved from the national database of identification (SANITEL), the number of herds in which at least one birth occurred between 01/01/2015 and 30/09/2015 and the number of births were determined.

Information on laboratory analyzes and official individual BVD statutes were obtained from the national BVD database.

A PI animal was defined as a bovine having official status "PI" on 11/30/2015. This status is assigned when a bovine gets at least one positive result and no negative result afterwards.

The incidence of BVDV at birth was estimated by dividing the number of PI calves born during the examined period by the number of calves born and tested at birth during the same period.

The herd prevalence was obtained by dividing the number of herds where at least one PI calf was born during the examined period by the total number of herds where at least one birth was recorded in the same period.

Herd prevalence and birth incidence were estimated with 95% confidence intervals (95% CI) assuming a binomial exact distribution.

Statistical analysis of the data was performed using a chi-square test (Toma B. et al., 2001).

Results: The biggest part of the sampling at birth (99.4%) was achieved by using ear tags associated with ear biopsy Allflex ULTRA-TST.

0.56% of the samples performed in this way have failed, leading to the reception of an empty trocar in the laboratory. Same observations were made in Ireland (0.44%; D. Graham, unpublished).

0.53% (95% CI: 0.51-0.55) of the calves born during the period were detected PI.

In comparison, the proportion of PI calves detected at birth in Switzerland at the start of the eradication plan was 1.8% (Presi P. et al., 2011). The incidence of BVD at birth observed in Wallonia seems more favorable but remains comparable to those reported in Lower Saxony (Brackmann J. et al., 2014) and Ireland (Graham D., unpublished), which were respectively 0.75 % and 0.61%.

The proportion of herds faced with the birth of at least one PI calf during the period was 9.45% (95% CI: 8.84-10.06). This herd prevalence is lower than the estimates made before the plan and based on a serological approach by Sarrazin S. et al. (2013) which concluded that 44% of Belgian herds were faced with the presence of PI animals.

In infected herds, between 1 and 20 PI calves were born during the period. In 95% of cases, the number of detected PI was less than or equal to 7.

Only 7.2% of dams of PI calves tested were themselves PI.

Herd size with a number of more than 100 animals was significantly associated with risk of PI calves birth (OR = 3.13; 95% IC: 2.66-3.67; p <0.001).

Conclusions: The first results of the BVDV eradication plan implemented in the southern part of Belgium are encouraging. Technically, farmers seem to really master ear-notch sampling linked with primary identification tag. Moreover the proportion of born PI calves and the proportion of infected herds observed in the first nine months are lower than preliminary estimates. However, these first elements do not allow us to predict the effectiveness of the plan or the time required to achieve a complete remediation of the Belgian livestock.

BVD

P03-003-044

The consequences of a bovine viral diarrhoea virus outbreak on herd level production parameters, a case study

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Objectives: Detecting the circulation of the Bovine Viral Diarrhoea virus (BVDV) in a dairy herd merely based on clinical signs is difficult and often impossible. As a result, recognizing BVD solitary by clinical presentations is a challenge for the bovine practitioner. Proving subclinical damage by BVDV is even more problematic. It has been described earlier that transiently BVDV-infected herds may have higher bulk milk somatic cell counts (SCC) compared with herds considered BVDV-free. The objective of the present case study was to visualize the effect of a confirmed BVDV outbreak on herd level production parameters.

Materials and Methods: The BVDV infection at the herd level was confirmed by screening for BVDV-Ag via a bulk milk sample. In addition, all animals older than three months and not included in the bulk milk sample were blood sampled for BVDV-Ag detection. Blood samples were pooled per 20 animals and RT-PCR was used for virus detection. If the pooled sample turned out to be positive, a BVDV-Ag ELISA test at the animal level was performed. In case an animal was found positive for BVDV antigen, the animal was retested 3 weeks later via a BVDV-Ag ELISA blood test. Fertility data were extracted from the farmer's herd management software. Milk production and somatic cell count (SCC) data were obtained via the 6-weekly milk recording. Three time periods were used in the case study. First, the period nine months preceding the birth of the oldest persistently infected animal detected during screening. The second time period was defined as the period when the detected PI's were on farm. The last period started after the culling of the last PI detected during the screening. Following parameters were calculated per period: average herd milk SCC, average herd milk production, average conception rate and average number of inseminations per pregnant cow.

Results: BVD antigen samples were taken following a BVDV positive abortion on a dairy farm in January 2014. The bulk milk sample of 138 lactating cows turned out to be negative. In total 208 animals were blood sampled for BVDV-Ag detection. Eight animals appeared to be positive. Five animals were confirmed to be a PI and 2 animals turned out to be transiently infected after retesting. One animal died before the second test could be performed. The first PI, detected during screening, was born in the beginning of July 2013. All five PI's were culled at the end of January 2014. The time period prior to the presence of the PI's detected during screening, ran from the first of October 2012 to the end of June 2013. The time period after the culling of the detected PI's ran from the end of January 2014 to the end of October 2014. During the three periods, the average daily milk production per cow decreased from 27.7l to 24l and increased again to 25.5l after the last PI was removed. The average herd SCC raised from 221 to 293 and subsequently declined to 269 once the PI's had been removed. One month prior to the birth of the first detected PI, the average herd SCC was already elevated to 308. Only the month after the birth of the first PI the average herd SCC was higher. The conception rate lowered from 45% to 35% and increased back to 44%. The number of inseminations per pregnant cow was 1.96 in the period prior to the presence of the PI's. It elevated to 2.16 during the BVDV outbreak and dropped to 2.08 after the culling of the PI's.

Conclusions: Via data analysis we showed that the average herd SCC increased, milk production dropped and fertility declined when PI animals were present in a dairy herd. Clearly, not all deteriorations in herd production parameters can be attributed simply to the presence of PI animals. Other factors such as: ration changes, average days in milk and alterations in the management will have an effect on herd production parameters as well. It can be concluded that by combining production and fertility data with BVDV test results, the veterinary practitioner can demonstrate the adverse effects of the BVDV on herd health and production.

BVD

P03-003-045

Identification of a new emerging strain of Border Disease Virus (BDV-8) in Italy using an antigen capture ELISA and real-time PCR.

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Objectives: Recently a Border disease strain (BDV) from a goat and a chamois (*Rupicapra rupicapra*) has been reported in northwestern Italy. Phylogenetic analysis based on the combined 5'-UTR and Npro/partial C genes, showed divergence from known BDV genotypes, revealing the detection of a novel pestivirus group, named BDV genotype 8. Mutations in the 5'-UTR of this emerging strain rises the concerns about the "diagnostic escape" that could occur during routine investigations.

In this study an antigen capture based ELISA kit and a commercial real-time PCR test for BVDV and BDV were used to define the level of detection of the new BDV-8 strain.

Materials and Methods: The IDEXX RealPCR BVDV RNA Test is a real-time PCR test detecting BVDV-1, BVDV-2, BVDV-3 (Hobi-like pestivirus) and BDV. The test works on a modular platform, where a shared RNA Master Mix and a pooled positive control can be used with any RealPCR RNA test. The IDEXX BVDV Ag/Serum Plus Test is an ELISA test based on the detection of BVDV E^{ms} antigen.

A panel of spleen, lung and ear notch samples were prepared from bovine, goat and chamois for real-time PCR analysis. The total sample amount was 8 samples. The samples were collected from animals recently infected with the new BDV-8 and with BDV-3 strains; BVDV-1b, BVDV-1e and BVDV-1f were also included in the study. For ELISA testing, ear notches, spleen and lung infected with BDV-8 and BDV-8 were prepared using the ear notch lysis buffer. The testing followed the manufacturer's instructions.

Results: Both tests showed good sensitivity on all sample types tested. One sample tested negative with the RealPCR BVDV RNA Test, most likely as a consequence of RNA degradation resulting from multiple freeze-thaw cycles. In addition to correct identification of the new BDV-8 strain and the BDV-3 strain, the real-time PCR test was also able to identify BVDV-1b, BVDV-1e and BVDV-1f strains also included in the study.

Both BDV-8 and BDV-3 strains were correctly identified with the BVDV Ag/Serum Plus Test.

Conclusions: This study shows that both ELISA and real-time PCR tests from IDEXX have a broad strain coverage for BVDV and BDV and are able to detect the new BDV-8 strains recently discovered in northwestern Italy.

BVD

P03-003-046

Demonstrating the efficacy of good biosecurity for BVD control on UK cattle farms

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Objectives: A lack of proof for the efficacy of biosecurity measures to control disease has been identified as a major barrier to the promotion and implementation of biosecurity practices by vets and farmers. The aim of the study was to assess the efficacy of on-farm biosecurity to protect cattle from BVD infection.

Materials and Methods: Blood samples were collected from 24,983 cattle on thirty-nine farms in Scotland and Northern England to assess BVD status. The nine veterinary surgeons for these farms then completed a questionnaire and semi-structured interview on their clients' biosecurity practices. Biosecurity on each farm was classified as poor, fair or good on the basis of these data. Mixed effects models were used to explore the association between biosecurity and BVD status at both farm and animal level while also accounting for covariates. The relative contribution of each of the risk factors to the within-herd seroprevalence and average herd-level BVDV antibody level was quantified after adjusting for the effects of other factors.

Results: Farms where biosecurity practices were classified as good had significantly lower BVD seroprevalence and mean antibody titres than farms which were classified as poor or fair. Animals on farms with poor biosecurity practices are five times more likely to be seropositive for BVD than animals on farms with good biosecurity. On the farms with good biosecurity, mean antibody titres remained low over the four years of the study but increased on the farms with poor or fair biosecurity standards. Cattle on beef farms were twelve times more likely to test seropositive for BVD than cattle on dairy farms, and cattle from large herds were twice as likely to be seropositive when compared to cattle from small herds.

Conclusions: The results provide evidence for the efficacy of biosecurity measures to control BVD infection on cattle farms in the north of Great Britain. The study also indicates that veterinary surgeons' assessments of their clients' biosecurity practices are accurate predictors of the effectiveness of these measures to control disease.

BVD

P03-003-047

The production impact of an acute BVD outbreak and the economic evaluation of BVD vaccination on a large-scale Hungarian dairy farm – A case study

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Objectives: Bovine viral diarrhoea (BVD) virus has a prominent importance among the infectious agents of cattle, due to its direct (production losses) and indirect (trade restrictions) effects on the industry. The average economic loss attributable to BVD was estimated to be € 12.6 per cow in Hungary in 2013. An acute BVD outbreak was estimated to cause € 155k damage for a dairy farm with 1,000 cows, which would suffer € 10k yearly loss due to chronic subclinical infection. The aim of our study was to present the production impact of an acute BVD outbreak and the economic evaluation of BVD vaccination in a large-scale Holstein-Friesian dairy herd.

Materials and Methods: The study was carried out in an averagely 1070-cow Hungarian dairy herd with an average 305-day milk yield of 8,593 kg between 2010 and 2012. In 2010 the number of abortions and calves' culling and mortality rate increased on the farm unexpectedly and significantly. The BVDV infection was confirmed in summer 2011 and in autumn 2011 the BVD vaccination (Bovilis® BVD, MSD-AH) was started in the herd. The major production and animal health parameters on the farm were surveyed between 2010 and 2012. Thus, we compared the production indices influenced by the BVD outbreak (abortion rate, calves' mortality and culling rate) in the year before the vaccination (2010) with those in the year when the entire herd was vaccinated against BVD (2012) in order to conduct the cost-benefit analysis of the BVD vaccination. In the economic analysis the reduction of losses due to the improvement of the BVD-affected production parameters was considered as benefit and the costs of vaccination were taken into account as cost. The average costs and prices of the study period were used in the calculations, where 1 EUR = 300 HUF (value of a 7-day old calf: € 66.67, raising cost of a 6-month old calf: € 426.67, salvage value of a culled calf: € 287, price of BVD vaccine: € 2.61/dose).

Results: The results show that the acute BVD outbreak had a strong detrimental impact on many production and herd health parameters, but after vaccination significant improvement was observed. Between 2010 and 2012 the abortion rate decreased from 4.52% to 1.55%, the rate of newborn calves with hydrocephalus from 0.6% to 0.0%, the mortality rate in suckling calves (up to 60 days of age) from 7.19% to 3.76%, the disposal (mortality and culling) rate in calves aged between 3 and 6 months from 3.49% to 1.92%, the disposal rate in heifers aged between 7 months and 1 year from 2.36% to 0.52%, and the mortality rate in cows from 0.90% to 0.69%, respectively. The number and rate of curative treatments in calves and heifers up to 1 year of age dropped from 427 (57.16%) to 233 (25.75%) cases. In 2012 compared to 2010 the number of digestive treatments in calves and heifers diminished by more than 50% (321 vs. 157 cases), and that of respiratory ones with almost 30% (106 vs. 76 cases).

The annual loss caused by the acute BVD outbreak was calculated to be 24.3 € per cow in 2010. The impact of BVD on milk production was excluded from the loss calculation, because it could largely be influenced by other production factors. In the herd the annual cost of BVD vaccination was € 11,223, but the decrease in the loss owing to an acute BVD outbreak amounted to € 20,240, which means € 9,017 extra profit yearly. The BVD vaccination yielded a profit of 7.7 € per cow in 2012, so as an investment it had 1.8 benefit-cost ratio and 80.35% ROI.

Conclusions: The findings of the case study show that an acute BVD outbreak can bring about significant economic losses, especially in BVD naïve herds. According to the result of the economic analysis, the BVD vaccination was highly profitable, since the benefits stemming from the improved herd production and health parameters exceeded the vaccination cost by far. In herds experiencing substantial losses due to BVDV infection, a vaccination program is worth considering from an economic point of view.

Objectives: This case report will discuss the pitfalls of BVD control management on a Dutch dairy farm where BVD re-entered the farm after several years of being "BVD virus free" and vaccination. Recommendations for improvement are proposed.

Materials and Methods: The farm consisted of a dairy herd containing 170 Holstein Friesian dairy cows with an average milk yield of 7219 kg milk/year with approximately 160 young stock. In summer time cows grazed on pasture from April until October close to another farm. In wintertime, these animals were kept indoors on a ration of grass- and maize silage and minerals.

Vaccination with an inactivated BVD vaccine started in 2008. At that time no information on the BVD status of the herd was available. Vaccination with the inactivated vaccine was performed twice a year according to the label recommendations in 2008 and 2009. Afterwards vaccination with the same vaccine was done only once a year in the adult animals until March 2014. The young stock was vaccinated twice with a 4 week interval. Other vaccinations carried out were monovalent IBR vaccines in cows and multivalent vaccines for pneumonia in young stock. Pneumonia had been a problem in calves for years but the incidence had decreased after improvement of the colostrum management and vaccination. Only a heifer breeding bull was purchased. Measures to monitor the introduction of BVD was done occasionally by blood sampling young stock for antibodies. There was no indication of BVD virus circulation, therefore the farm was considered to be BVD virus free.

Results: In the summer of 2015 pneumonia was diagnosed based on clinical symptoms (coughing, dyspnoea and fever) in calves of 2 to 4 months old. Older calves of 5 to 6 months of age had Trichophyton infection with severe clinical symptoms. Immunosuppression was suspected and diagnostic testing for BVD was performed. Five blood samples from young stock were antibody positive. The whole herd was sampled via bulk milk and individual blood samples for the presence of BVDV. Two persistent infected (PI) animals (born December 2013 and June 2015) were identified and removed. By the age of the oldest PI it was calculated that the BVD circulation took place since the summer of 2013. At the neighbouring farm BVD was also diagnosed in the beginning of 2014. Both PI animals were infected with BVDV type I. All new born calves are tested for BVDV since. So far one new born calf was found to be a PI animal. After removal of the PI's vaccination continued with a new live BVDV vaccine (Bovela, Boehringer Ingelheim) with a 12 month duration of immunity.

Conclusions: On this farm BVD control had been inadequate: no identification and removal of PI and a vaccination interval beyond the 6 month duration of immunity of the inactivated vaccine. To conclude, this case reports emerges the fact that when BVDV control is inadequate all the implemented strategies can be a waste of effort and money. In order to prevent BVDV circulation and (clinical) problems a close collaboration between veterinarians and farmers is needed. Vaccination of all animals from 3 months of age with a live BVD vaccine with a 12 month duration of immunity can help the vet and the farmer with an easier to implement vaccination strategy.

BVD

P03-003-048

BVDV Infection: a never ending story! A case report about the consequences of pitfalls in BVDV control.

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BVD

P03-003-049

Reproductive outcomes in pregnant ewes experimentally infected with bovine viral diarrhoea virus between days 59 and day 69 of gestation

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Objectives: Bovine viral diarrhoea virus (BVDV) is an important and highly prevalent disease of cattle associated with immunosuppression and reproductive losses. Many non-bovine species, including sheep, pigs, camels and deer, have been shown to be susceptible to infection with BVDV, with similar outcomes to those seen in cattle. The aim of this study was to determine what reproductive outcomes would result following the infection of ewes with an Australian BVDV strain between 59 and 69 days gestation.

Materials and Methods: Twenty two ewes, pregnant with 42 foetuses, were inoculated with 2ml of BVDV infected cattle serum at 59-69 days gestation. The remaining 11 ewes, pregnant with 23 foetuses, were not inoculated. Blood samples were taken weekly from all ewes until the week prior to lambing. Pregnancy scanning was undertaken fortnightly, on five occasions. All ewes were fed under the same conditions, although housed separately. Blood samples were obtained from lambs born to infected ewes on the day of birth and weekly until eight weeks of age and an ear notch sample was obtained on the day of birth. Dead, stillborn or aborted foetuses/lambs of infected ewes were submitted for post mortem examination. Serum samples were analysed for BVDV-specific antibodies and BVDV viral antigen by ELISA.

Results: All 22 infected ewes showed seroconversion to BVDV by 49 days post infection. No other signs of infection were observed in any of the ewes. All 11 control ewes remained naïve.

High reproductive losses were observed in the infected ewe flock compared to the control ewes with foetal losses being 83% and 17% respectively. Post mortem examination of aborted, stillborn and dead lambs from infected ewes revealed severe abnormalities including anasarca, pleural effusion, arthrogryposis, severe skull deformities, hydrancephaly and hydronephrosis. One lamb, which subsequently died, presented as a 'hairy shaker' with an abnormal hair coat and muscular tremors. All lambs from infected ewes were of small size and birthweight. One of the 4 surviving lambs was persistently infected with BVDV, surviving until euthanasia at eight weeks of age.

Conclusions: This study has shown that infection of pregnant ewes with BVDV between days 59 and 69 of gestation can result in high foetal losses, many with severe abnormalities. It has also shown that viable persistently BVDV infected lambs can be produced following infection with BVDV between 59 and 69 days gestation. However, the potential for these persistently BVDV infected lambs to infect naïve animals, particularly sheep and cattle, needs to be further studied in order to determine what risk sheep populations may play in the epidemiology of BVDV.

BVD

P03-003-050

Transmission of Bovine Viral Diarrhoea Virus from a persistently infected lamb to naïve sheep

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Objectives: Typically associated with cattle, Bovine viral diarrhoea virus (BVDV) has been shown to cross-species barriers and infect a wide range of ruminant and pseudo-ruminant species including sheep, goats, alpaca and deer. Infection with BVDV can lead to immunosuppression and a wide range of reproductive losses resulting in severe economic losses to farmers. The aim of this study was to determine if naïve sheep would seroconvert to BVDV following co-mingling with a persistently BVDV infected (PI) lamb.

Materials and Methods: Four sentinel BVDV naïve ewes and their five sentinel BVDV naïve lambs were mixed with a flock of recently lambed ewes which had been experimentally infected with BVDV between 59 and 69 days gestation. One viable and confirmed BVDV PI lamb was present in the infected ewe flock. The sentinel animals remained with the infected flock for a period of eight weeks at which time the PI lamb was euthanized. The sentinel animals were blood sampled weekly for the eight weeks following the introduction to the flock and then on two occasions, a fortnight apart, following euthanasia of the PI lamb. Serum samples were analysed for the presence of BVDV-specific antibodies.

Blood samples were taken from the PI lamb from birth until euthanasia at eight weeks of age. Serum samples were analysed for BVDV-specific antibodies and BVDV-specific antigen by ELISA.

An additional control group of 6 BVDV naïve ewes and their 8 lambs were kept in a paddock with a 3m separation from the flock with the PI lamb for a total of 8 weeks. Blood samples were taken from the control group at the end of this period. Serum samples were analysed for BVDV-specific antibodies by ELISA.

Results: Seroconversion to BVDV was observed in one naïve lamb at seven weeks post mixing and then again at eight weeks post mixing. At this eight week time point no other sentinel animals were shown to have sero-converted.

Serum samples taken from the PI lamb indicated the presence of colostral BVDV-specific antibodies at all time points. However, a strong positive antigen result was present on day of birth and again from week 2 to week 8.

All the control animals returned negative test results for BVDV-specific antibodies.

Conclusions: This study has shown that seroconversion in naïve sheep can occur following co-mingling with a BVDV PI lamb. It is thought that the low rate of seroconversion in these naïve animals is due to the inhibitory influence colostral antibodies have on viral excretion.

This study has also demonstrated that no infection occurred in sheep kept in a paddock with a 3m separation gap from the paddock containing the PI lamb.

BVD

P03-003-051

BVD Foetal Protection Induced By Vaccination In Cattle

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Objectives: The following trial was undertaken to determine if vaccination of sero-negative heifers with a multivalent viral vaccine (HIPRABOVIS®, "Hipra"; Spain) containing a 1a Bovine Viral Diarrhoea (BVD) strain was

able to protect the foetus from infection with a field strain of BVDV. This study was conducted by: ESTENDART LTD, Palmerston North, New-Zealand.

Materials and Methods: Forty eight non-pregnant heifers seronegative to BVDV, Neospora caninum and Enzootic Bovine Leukosis were randomly allocated to two groups of 24. Heifers in one group were vaccinated with a multivalent viral vaccine (HIPRABOVIS®) followed by a booster 21 days later. Heifers in the second group were unvaccinated controls. All 48 heifers were oestrus-synchronized in a CIDR programme then mated using artificial insemination. Ten heifers from each group shown to be pregnant were selected 66 days after insemination and grazed together with 2 persistently infected (PI) calves for the remainder of gestation. All calves born alive were blood sampled at birth prior to suckling colostrum and their BVD status determined by serum neutralization test (SNT) and ELISA Antigen.

Results: Six heifers (1 from the vaccinated group and 5 from the controls) had late abortions (calves at term). All 6 calves were positive for BVD by ELISA Antigen and antibody. Eleven calves were born alive: 7 in the vaccinated group and 4 in the controls. Two of 7 live calves in the vaccinated group were identified as PIs at birth (28.5%) whereas the 4 live calves in controls were all identified as PI animals (100%). When late abortion and PI calf numbers were combined, the protective effect of vaccination with the multivalent viral vaccine (HIPRABOVIS®) was significant compared with the unvaccinated group ($p=0.009$).

Conclusions: The results demonstrate that the use of the 1a BVD strain of a multivalent viral vaccine (HIPRABOVIS®) in seronegative heifers provided significant protection against foetal infection when the herd was exposed to a high level of natural BVD challenge during gestation.

BVD

P03-003-052

Impact of administration of two inactivated bovine viral diarrhoea virus (BVDV) vaccines on serological test results in dairy heifers

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Objectives: BVD vaccines are an integral component of viral control on-farm. Use of BVDV vaccines, however, is a potential confounding factor when interpreting BVD serological results. Previous international studies have examined the impact of vaccination against BVDV on individual animal antibody responses to p80 (non-structural protein 3) and whole virus (WV) antigens, with equivocal results. The aim of the present study was to undertake a controlled trial to investigate the impact of BVD vaccination on the serological status of seronegative dairy heifers using p80 and WV antibody ELISAs.

Materials and Methods: Two inactivated BVD vaccines (Bovidec BVD, Novartis Animal Health and Bovilis BVD, MSD Animal Health) were administered to two treatment groups of 35 yearling maiden heifers. Group sample size was determined to yield a minimum power of 0.08

and a 5% error. A 'before and after' study design was used with animals acting as their own controls. Heifers were assigned to treatment groups based on the vaccine to be administered and balanced by breed (purebred Holstein-Friesian versus crossbred) and body weight. All heifers were blood sampled prior to vaccination. A primary vaccination course (i.e. primary dose and booster at an interval specified by each manufacturer) was administered to each heifer. Heifers were re-sampled at day 14, 28, 60, and 180 post-vaccination. Samples were tested using seven commercially available BVD ELISA kits. Of these, five detected antibodies against p80 antigen and two detected WV responses.

Descriptive data were coded and analysed in Excel (MS Office 2010). ELISA results were categorised as positive or negative. Doubtful results were also classified as negative. Statistical analyses were completed using Stata version 12 (StataCorp, US). Differences across treatment groups and between ELISA kits over time were investigated general estimating equations. A binomial distribution, logit link function, and an autoregressive correlation were assumed. Results were reported as exponentiated coefficients (odds ratios).

Results: All 70 heifers tested seronegative pre-vaccination on each of the ELISA tests used with the exception of a single test kit (a confirmatory WVELISA) which recorded eight seropositive individuals. By day 14 post-vaccination, all test kits yielded seropositive individuals amongst the 70 tested in both treatment groups (range 7-36, p80 kits; range 65-68, WV kits). By day 180 post-vaccination, the number of seropositive heifers, and the likelihood of recording a seropositive result, decreased significantly ($P<0.001$) across all test kits (range 4-27, p80; range 13-26, WV). When all test results were considered, heifers vaccinated with Bovidec were almost three times more likely ($OR=2.9$; $P<0.001$) to record a seropositive result than those vaccinated with Bovilis over the duration of the study. The odds ratio of recording a positive result post-vaccination compared to pre-vaccination decreased from 274:1 at day 14 to 23:1 at day 180 ($P<0.001$) for Bovidec-vaccinated heifers and from 136:1 at day 14 to 24:1 at day 180 for Bovilis-vaccinated herds.

Conclusions: Administration of inactivated BVD vaccines impacts on interpretation of individual BVD serological results. The extent of confounding, however, varies significantly across vaccine brand, ELISA test kit, and the date of sampling post-vaccination. In general, use of p80 kits will facilitate interpretation of ELISA results in vaccinated herds. Based on the results of this study, it may be possible to identify a combination of inactivated vaccine and p80 ELISA which would allow use of BVD serological surveillance in a vaccinated population.

BVD

P03-003-053

Serological evidence of HoBi-like virus circulation in Argentinean water buffaloes

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Objectives: The aim of this work was to determine and compare the antibody levels against BVDV-1, BVDV-2 and HoBi-like virus in non-vaccinated water buffaloes from three provinces in the Northeast region of Argentina.

Materials and Methods: Mediterranean and Murrah Water buffaloes (n = 130) were sampled from 12 extensive breeding farms across Corrientes, Chaco and Formosa provinces in Argentina. Blood samples from each animal were aseptically collected from the jugular vein in sterile Vacutainer using separate needles. [FB1] Sera were obtained after centrifugation and the inactivation was performed at 56°C for 30 minutes.

Virus neutralization assays were performed for each serum, using 96-well plates, MDBK cells and 100 TCID₅₀ of cytopathic BVDV-1-Singer, BVDV-2-VS253 and a HoBi-like virus isolate. Two-fold serial dilutions were performed in the serum samples to determine the neutralizing antibody titers against BVDV-1, BVDV-2 and HoBi-like virus. The neutralizing antibody titers were expressed as the reciprocal of the highest dilution that neutralized viral infectivity. Spearman-Kärber titers were calculated to analyze the results.

Results: While eighty-eight water buffaloes were seronegative to the three pestiviruses, 53% of the seropositive animals (n=22) contained significant higher levels of neutralizing antibodies against HoBi-like virus than to BVDV-1a or BVDV-2. About 40% of the seropositive animals showed significant higher levels to BVDV-1 in comparison to BVDV-2 and HoBi-like virus and the rest of them (7%) showed similar neutralizing antibody levels against BVDV-1 and HoBi-like virus.

All the animals that showed higher neutralizing antibody titers against HoBi-like virus belonged to farms located in Corrientes Province, border with Brazil. On the other hand, water buffaloes from Formosa and Chaco provinces were seronegative against the three strains or showed higher levels of neutralizing antibodies against BVDV-1.

Conclusions: The circulation of BVDV-1 in argentinean water buffaloes has been previously reported (Craig et al, 2015). However, in this work, we found for the first time serological evidence of HoBi-like virus circulation in water buffalo livestock in Argentina. The provinces involved in this study are located near Brazil, where several reports of HoBi-like virus in cattle herds were disclosed.

Further work should be performed to isolate the local HoBi-like virus and to determine its pathogenicity. Also, it should be determined if this virus is also affecting cattle from the analyzed region.

BVD

P03-003-054

Prevalence of bovine viral diarrhoea virus infection in Hokkaido, Japan; epidemiological analyses based on the viral genotype

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Objectives: Persistently infected (PI) cattle with bovine viral diarrhoea virus (BVDV) spread a lot of virus particles within the herd. When the fetus infect with BVDV in uterus at some pregnancy period, PI calf is produced at the results of immunotolerance. PI cow is the central issue for the control of BVDV as the source of infection. The estimation of the prevalence status of BVDV is important for the control of BVDV infection. In the present study, we investigated the virus genotypes of PIs detected in Hokkaido, Japan, during 8 months period, and analyzed epidemiologically.

Materials and Methods: From November 2014 to July 2015, approximately 26,000 cattle were examined for BVDV infection in Veterinary Teaching Hospital of Rakuno Gakuen University, and 124 PIs were detected. Viral gene was detected from their sera by reverse transcription polymerase chain reaction (RT-PCR) using specific universal primers for BVDV gene. For the classification of the genotype as BVDV1 or BVDV2, RT-PCR using genotype specific primers was utilized. The relationships between genotypes and species, gender, age, occurrence place, or infected place were estimated.

Results: The ratio of BVDV1 and BVDV2 was 7 (88 PIs): 3 (36 PIs). The deviation of genotype distribution pattern was almost the same with previous report in Japan. Only 3 PIs indicated clinical symptoms such as pneumonia or diarrhea. The virus genotype distribution in female and male PIs was almost the same in each gender. There was no different distribution of genotype in three species, Holstein, Japanese Black and F1, and also not in different age. In occurrence place of PI, BVDV2 significantly dominant (p<0.05) area was recognized. The obvious cause of this dominance was not clear.

Conclusions: The deviation of prevalent genotype in BVDV detected during short period (8months) was almost the same with previous report. This suggested that vaccination control is not effective for BVDV PI over a decade.

BVD

P03-003-055

Serological and molecular confirmation of bovine viral diarrhoea virus subgenotype 1b in yearling buffaloes (Bubalus bubalis) from Northeastern Brazil

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Objectives: Bovine viral diarrhoea (BVD) is an infectious disease that affects cattle of all ages, that is caused by bovine viral diarrhoea virus (BVDV), and occurs worldwide. The susceptibility of buffaloes (Bubalus bubalis) to this virus was demonstrated by serology and molecular techniques. Although there are serological demonstrations of BVDV in buffaloes from Brazil, there is no molecular confirmation of the viral subgenotype circulating in buffalo herds. The objectives of this study were to describe the serological prevalence of BVDV-1 in buffaloes and determine the molecular subtype circulating in herds from Maranhão state, Northeastern Brazil.

Materials and Methods: This study was performed in two buffalo farms (A, n=316; B, n=387), located in the state of Maranhão, Northeastern Brazil. Buffaloes at these herds were reared in close proximity and had sporadic contact with cattle. Serum samples (n=223) from asymptomatic yearlings buffaloes were collected (herd A, n=105; herd B, n=118). These samples were submitted to the BVDV-1a (Singer strain) virus neutralization (VN) technique according to OIE. To determine which genotype of BVDV was circulating in each herd, the nucleic acid of BVDV was extracted from serum samples and used in RT-PCR assays designed to amplify the 5'UTR region of BVDV by using the 324/326 primers. Positive and negative controls were included in each RT-PCR assay. Selected positive serum samples were then sequenced and the derived amplicon analyzed phylogenetically to determine the relationship with similar isolates.

Results: Serological analyses revealed that 40% (42/105) of the yearlings at herd A were seropositive to BVDV-1, with seropositivity being 52.5% (62/118) at herd B. The presence of anti-BVDV antibodies at these two buffalo herds confirmed the circulation of this virus at these locations. However, from the 62 serum samples (herd A, n=52; B, n=10) that were subjected to the BVDV RT-PCR assay, 17.7% (herd A, n=6; B, n=5) were viremic at that time and were considered as being transiently infected. Additionally, sequencing and phylogenetic analysis demonstrated that two isolates clustered with other strains of the BVDV 1b subgenotype.

Conclusions: The results of the serological assays demonstrated that a large proportion of buffalos at both herds were infected by BVDV-1. Additionally, the amplifying and sequencing of BVDV from two seropositive animals confirmed that these were infected by this virus, while phylogenetic relationship revealed that these yearling buffalos were infected by BVDV 1b subgenotype. Additionally, the presence of the BVDV in these buffalos was probably due to the close contact with cattle. Although buffalos are rustic and considered resistant to several diseases, in this case the buffalos were susceptible to infection by BVDV.

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BVD

P03-003-056

The use of Clinical Audit to support structured communication and diagnostic protocols in BVDV control across a national veterinary practice

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Objectives: A farm's Bovine Viral Diarrhoea Virus (BVDV) status can be clearly determined by applying systematic diagnostic protocols as part of (or outside) accredited health scheme protocols, allowing the targeted implementation of appropriate disease control measures. The impact of a structured communication and engagement campaign on farm engagement with BVD control was assessed in a large veterinary practice using paired clinical audits.

Materials and Methods: A clinical audit of farm/herd BVD status was conducted in August 2014, across a multi-site national farm animal veterinary group in dairy farms in advance of a structured communications and engagement campaign of regional meetings, internal webinars and newsletters/leaflets to farmers over a 3 month period in Spring 2015. The clinical audit was repeated 16 months later. Farm BVDV status was assigned in line with health scheme definitions (CHECs protocols), and the level of engagement in BVDV control was defined by the level of engagement with systematic BVDV diagnostics used (members of health scheme; structured testing outside of health scheme; no/sporadic testing). The proportion of farms engaged in health schemes and structured testing was compared, as were changes in herd status for BVDV.

Results: The initial clinical audit revealed inconsistencies in the way vets were classifying farms BVD status, though these could easily be rationalised by retrospectively applying health scheme definitions of farm status. The second clinical audit will be conducted in the winter of 2015/2016 using health scheme definitions of BVDV status. Comparisons between the two audits will include changes in the number of farms engaged with structured BVDV testing/control (either within our

outside health schemes), changes in the health status of farms (both within and outside health schemes), and the results will determine the value of the structured communication and engagement campaign in driving engagement with BVDV disease control on-farm.

Conclusions: This study does show the benefit and importance of working alongside CHECs recommendations and health scheme protocols. Standardising farm BVDV disease status classification improved communication/focus between participating veterinarians by establishing common language & definitions. Engaging farmers in structured discussions on BVDV control is likely to improve understanding of the disease impact, and buy-in to disease control. Clinical audit is a valuable tool in monitoring disease control, allowing focussed targeting of problem farms.

Comments: The authors are grateful for the support of Westpoint Veterinary Group's Infectious Disease Working Group, the Clinical Governance, Marketing and Knowledge Transfer Groups and Biobest Laboratories for their input into the conduct of this work.

BVD

P03-003-057

Relationship between initial BVD ELISA and real time RTPCR test values for ear tissue and the outcome of confirmatory testing of blood samples.

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Objectives: Currently all Irish calves are tested for BVD virus as part of a national eradication programme. After an initial positive or inconclusive result, a confirmatory test to differentiate between transiently infected (TI) and persistently infected (PI) animals may be performed on blood samples submitted to the National Reference Laboratory (NRL).

It was hypothesized that the test value associated with an initial positive or inconclusive result for an ear notch sample tested by ELISA or real time (rt)RTPCR, may be used to predict the outcome of a confirmatory test. The current study was undertaken to test this hypothesis.

Materials and Methods: Test results were retrieved from the programme database (managed by the Irish Cattle Breeding Federation) for all calves with an initial inconclusive or positive result on a tissue sample collected from the ear during using a modified identity tag that had also been subjected to a confirmatory test by the NRL. Calves were excluded if the inter-sample interval was <21 days.

For samples where the initial test was conducted by ELISA, the initial test values (S-N) were assigned to a series of incremental categories and the number and percentage of animals re-testing with inconclusive, positive or negative results determined for each category. Based on these numbers, the likelihood ratio (number positive: number negative) of animals in each category giving a positive re-test result was determined.

Data for samples where the initial test was conducted by rtRTPCR were analysed in a similar way.

Results: Results for a total of 21,181 calves met the criteria for inclusion. Of these, 17,498 and 3,683 had been tested initially by ELISA and rtRTPCR respectively. Results for the former indicated that the lowest likelihood ratios (0.20) were associated with those calves which had an initial inconclusive ($0.2 > S-N \leq 0.3$), or a weakly positive ($0.3 > S-N$

≤0.4) ELISA result. The ratio increased with S-N values ($r^2= 0.967$, $y = 3.1676x^{2.513}$). Values of 1.0 and above had consistently high likelihood ratios (4.0-76.5). A similar pattern was evident when rtRTPCR results were analysed, with Ct values on initial testing of >30 being associated with low likelihood ratios (≤0.2), whereas those with lower Ct values I had a high likelihood of being PI, with ratios in the range 3.0 to 27.8.

Conclusions: The initial test value obtained by ELISA or RT-PCR on ear punch can be used as an indicator of the likelihood that the calf will be positive on re-test (and therefore PI). This information can be used to assist decisions as to whether or to re-sample a given calf and help ensure the prompt removal of PI calves.

BVD

P03-003-058

Do Too Many Tests Spoil the Eradication of Bovine Viral Diarrhoea?

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Objectives: Globally, the eradication of bovine viral diarrhoea is still in its infancy but eradication has been, or is being, adopted by several countries. Lessons can be learnt from this progress in two main ways. The first is by making comparisons between countries to assess best practice and the second is by pooling published results from different countries to get greater statistical power in asking scientific questions of the data. We set out to evaluate whether a relationship between published BVDV test results could be created. We also examined the time taken to retest following an initial antibody BVDV test in the Scottish eradication scheme.

Materials and Methods: Differences in the way in which BVDV is measured are not just a barrier to academic progress, but also may affect the progress within an eradication scheme itself.

In an attempt to establish relationships between published BVDV test results we carried out a systematic literature review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

We tested the time taken to retest based type of initial antibody test using a one-sided proportion test. The samples were split by initial test type (blood or milk) and the proportions of those retesting within 90 days following an initial not negative result were examined.

Results: We report the results of the systematic review, providing a summary of papers where there is potential cross-calibration and a summary of the obstacles preventing such data-pooling. The main barriers came in the form of vaccination, test variety and the way test results were reported.

Graphical and statistical evidence from survival analysis show that within the Scottish BVDV eradication scheme, holdings retest quicker from a not negative antibody blood test compared to an initial not negative antibody milk test.

Conclusions: When an eradication scheme is being designed the tests and testing methods should be designed so the scheme does not discombobulate itself. Academic researchers can assist with this by considering how comparable their results are with existing test results.

BVD

P03-003-059

Control of bovine viral diarrhoea virus (BVDV) in Flores and Corvo Islands from the Azores archipelago

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Objectives: This project aims control and eradicate BVDV in cattle population from Flores and Corvo Islands, in the Azores through the screening and culling of persistently infected animals (PI). And still continue encourage the measures of biosecurity, the effective vaccination to prevent fetal infection in heifers and beef cattle and the consequences of BVDV infections in herds.

Materials and Methods: The Flores and Corvo Islands belong to the western group of the Azores archipelago. There are about 5000 bovines distributed by 60 herds from which 30 are dairy herds. In this survey all cattle from dairy herds were tested for the presence of antibodies against BVDV and for the presenc of BVDV. The screening was performed with individual serum samples using the enzyme linked immunosorbent assay (ELISA) antibody test and pools of serum and EDTA blood samples using Real Time Polymerase Chain Reaction (RT-qPCR).

Results: Seroprevalence and prevalence of BVDV was determined in 30 dairy herds, testing 227 cattle from Flores Island and 17 from Corvo Island, during October 2015. All the dairy herds were under official sanitation, most of them presenting fertility problems. It was not detect any transitory infected animal with BVDV and any PI animals. But the seroprevalence of BVDV between herds was 100% in Corvo Island and 84.0% in Flores Island. The seroprevalence of BVDV between animals was 46.5% and 82.35%, respectively.

Conclusions: This study was the first step to control and eradicate the BVDV in cattle from these two islands. Give the high rate of herds and animals affected by BVDV, it is essencial to take action in herds, do an epidemiological survey to find the cause of this seroprevalence, slaughtering PI animals, and implementing measures to prevent the dissemination of the virus in cattle population.

BVD

P03-003-060

Retrospective study of BVD 3 in Brazilian cattle herds

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Objectives: In 2004 Bovine Viral Diarrhea Virus 3 (BVDV 3) was detected from a batch of FBS and since then, the virus has been reapeated isolated from batches of FBS either from animals with respiratory signs or aborted fetus. BVDV belongs to the family Flaviviridae genus Pestivirus and is one of the most important pathogens responsible for reproductive fails and some cases are associated with respiratory and neurological

disorders. The aim of this study was evaluate the frequency of BVDV3 in Brazilian herds with neurological signs, reproductive fails or diarrhea from January 2005 to December 2014.

Materials and Methods: The material comprised samples received at laboratory of bovine viruses of Instituto Biológico of São Paulo State. They were from north, middle east, southeast and south regions of Brazil. A total of 246 samples were analyzed of which, 226 were batches of FBS, 174 were tissues from animals with neurological signs, respiratory or diarrhea sings and 18 aborted fetuses. The samples were submitted to RT-qPC using TaqMan system. Positive samples were subjected to sequencing using primers of 5'UTR region odified gene.

Results: The RT-qPCR detected BVDV in 34 FBS and 9 tissues (CNS, lung, faeces), totalizing 43 positive samples. BVDV was not found in fetus and diarrhea symptoms demonstrating other causes of abortion and gastroenteritis. From positive samples, 20 were submitted to sequencing and were identified genotype 3 in 16 samples (14 in FBS and 2 in tissues) and genotype 1 in 5 FBS, with approximately 95 to 99% of identity. The BVDV 3 was detected in Paraná (1), Sao Paulo (14) and Minas Gerais (1) States. On the other hand BVDV 1 was identified in Sao Paulo (4) and Parana (1) states.

Conclusions: The study highlights the importance of phylogenetic characterization of BVDV and demonstrate the circulation of BVDV 3 in Brazil, mainly in state of Sao Paulo and also the importance of monitoring batches of FBS.

Results: The data do not present normal distribution, so the statistic differences between medians of Abs (Log2) were determined by Kruskal-Wallis test. The correlation between Abs titers and age was performed by Spearman test. The Mann-Whitney test was used to evaluate the relationship between age and seropositive frequencies for p80. Heifers were seropositive (100%) of M1 to M13. Median Abs titers (Log2) found the M1 to M13, respectively, were 8.3; 7.3; 6.3; 5.3; 6.3; 5.3; 6.3; 6.3; 5.3; 6.3, 6.3 and 6.3. The Abs titers of obtained in M1 were different from the M2-M13 (P=0.001); M2 was statistic different M4 (P = 0.01) and M6 (0.05). The frequencies (%) seropositive heifers for p80 M1 to M13, respectively, were 24.7; 18.2; 10.8; 11.8; 73.3; 73.8; 72.4; 58.1; 45.9; 48.4; 46.2; 47.1 e 61.0. It was possible to verify correlation between Abs titers and ages, observing higher titers in younger heifers (P=0.001). Positive correlation was also observed for the seropositive rate in heifers with p80 from the M5 (P=0.001).

Conclusions: It is possible to conclude that maternal Abs titers showed gradual decline during the first five months of life, which coincided with the increase of seropositive heifers frequency for p80. The inversion occurred between the maternal Abs titers and increased Abs to p80 which indicates that the moment of greatest risk for natural infections caused by BVDV. It is believed that the immunosuppression caused by BVDV can be a risk factor for Respiratory Disease, Anapalsmosis and Babesiosis.

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BVD

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Interface Between Maternal Antibodies And Natural Challenge For Bovine Viral Diarrhea Virus (Bvdv) In Dairy Heifers

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Objectives: The objective of this research was to evaluate the interface between passive and active immunity for Bovine Viral Diarrhea Virus (BVDV) in Holstein black and white females in the first 13 months of age.

Materials and Methods: Serum samples were analyzed from 572 heifers by serum neutralization (SN) and indirect enzyme immunoassay (Elisa) for the p80 protein (BVD/BD p80/125 protein). The immunization program adopted was initiated with 1st milking colostrum intake (lg ≥ 50mg/mL), 4-6L in the first 12 hours of life, from cows vaccinated with the polyvalent vaccine Cattle Master Gold FP5+L5[®] (Zoetis, Brazil), which contains inactivated BVDV-1 (strain 5960) isolated of Iowa (USA) and BVDV-2 (strain 53637) an isolated of Ontario (USA). According to records of the farm, calves received the first dose of vaccine between three to ten months of age and a booster after 30 days, then, females were included in biannual vaccination schedule. Heifers were classified according to their age by the month of life: M1 (n=89), M2 (n=76), M3 (n=67), M4 (n=50), M5 (n=58) M6 (n=40), M7 (n=57), M8 (n= 30), M9 (n=35), M10 (n=28), M11 (n=13), M12 (n=16) and M13 (n=17). Serum samples were collected for specific antibodies (Abs) detection for BVDV by SN and Elisa (SERELISA BVD p80 Ab Mono blocking kit[®], Synbiotic).

Camelids

P02-002-212

Study on important viral infections in New World Camelids in Austria

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Objectives: The population of New World Camelids (NWCs) in Austria is now estimated to about 4000 to 6000. In a substantial number of herds in Austria the camelids are kept together with ruminants or they have indirect contact by being kept on the same pastures. NWCs are susceptible to a number of viral pathogens which are important for ruminants such as bovine herpes virus 1 (BHV-1), bovine viral diarrhoea virus (BVDV), Schmallenberg virus (SBV), bluetongue virus (BTV). The objective of the study was to assess the role of NWCs as potential carrier of certain viral pathogens and to estimate the potential infectious risk for ruminants.

Materials and Methods: The total number of NWCs in the study was estimated to be approximately 10% of the total population of NWCs in Austria. All federal states of Austria have been included in the study. The farms have been visited, management and husbandry evaluated, animals have been clinically examined and blood samples have been taken from the jugular vein. Further it has been recorded if the animals were born in Austria or imported from abroad. In the present study 447 serum samples from 186 llamas and 261 alpacas have been collected which were representative for entire Austria. The samples were examined for BHV-1 antibodies (Ab), for BVDV Ab and antigen (Ag), for SBV Ab, and for BTV Ab. Commercially available tests were modified and partially validated for NWC.

Results: From March to July 2014 78 farms have been visited and 447 animals older than one year have been examined and sampled; 186 of these animals were llamas and 261 alpacas. One sample was positive for BHV-1 Ab (0.2 %), one was positive for BVDV antibodies (0.2 %), zero were positive for BVDV Ag (0 %), 323 were positive for SBV Ab (67.7 %) and 53 samples were positive for BTV Ab (11.8 %). Age of the animals did not influence the frequency of AB detection. Differences between several regions of Austria have been found in BTV and SBV AB frequency which can be most likely explained by different geologic conditions (alpine mountains versus lowland areas). Additionally, tendencies have been identified that llamas show different infection pattern in comparison to alpacas. Data on prevalence of SBV, BTV, BVDV, and BHV-1 in NWCs had not been determined for Austria yet. The present study seems to be the first study, which describes the seroprevalence of these viruses in Austrian llamas and alpaca population.

Conclusions: Conducting such a seroprevalence study was important to assess the role of NWCs as potential carrier of viral pathogens and to estimate the potential infectious risk for farm animals especially ruminants. In summary it can be said that the two viruses BVDV and BHV-1 play currently a minor role in Austria. A high number of NWC were infected with BTV and SBV and have been found seropositive. It can be suggested that NWCs may act as a reservoir for BTV and SBV and may play an important role in its transmission. Therefore continuous monitoring of the population is necessary.

Camelids

P02-002-213

Transabdominal Ultrasound in South American Camelids: methods and physiological findings

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Objectives: There is a lack of information dealing with the method of abdominal ultrasonography in South American Camelids (SACs) and also the normal sonographic appearance of abdominal organs, a fact that seems to be basic prerequisite for the use of ultrasonography as a diagnostic tool in diseased animals. Only a few case reports describe abdominal diseases and their clinical pathology, using ultrasound as a diagnostic aid. Therefore the aim of this study was to establish an ultrasound method for examining different abdominal organs in SACs and to describe their normal ultrasonographic appearance.

Materials and Methods: The study was realised on private alpaca and llama farms in Austria. Parts of the abdomen of 100 clinically healthy adult llamas and alpacas (over 1 year of age) were examined ultrasonographically. For ultrasonographic examination the fleece of the animal was parted at the region of interest at the base of the hair and soaked with alcohol, before placing the probe on the skin. A 6.6 MHz convex transducer was used. The abdomen was scanned on the left and right side and in the ventral caudal region with attention to the kidneys, the urinary bladder, the spleen, the compartments, the duodenum, and the liver. Sonographic appearance of these organs and best location for their sonographic identification were described.

Results: Performing ultrasonography using only alcohol as contact medium between probe and skin without clipping the hair was performed successfully in all animals. On the left side of the abdomen the left kidney was best seen dorsally in the left paralumbar fossa between the 4th and 7th transverse process of the caudal lumbar vertebra. The hyperechoic renal pelvis, the hilus of kidney and the hypoechoic renal parenchyma could be visualised sonographically. The ureter was not visible. The basis of the spleen, with its homogeneous and echoic pattern displayed anterior to the left kidney and showed a typical L-form. Anechoic vessels inside the spleen and a thin echoic capsule surrounding the spleen tissue could be detected. Adjacent to the spleen the wall of the compartment 1 displayed as an echoic band and was localised cranial to the spleen. On the right side of the abdomen the liver was best viewed between the 9th, 10th and 11th intercostal space. The liver showed a hypoechoic, homogeneous tissue pattern with anechoic vessels inside. In the 10th intercostal space an ultrasonographic window allowed visualisation of parts of the right liver lobe, the echoic wall of the dorsal portion of the compartment 3 and parts of the duodenum. The right kidney was visible by positioning the probe in the right paralumbar fossa behind the last rib. For the visualisation of the urinary bladder the transducer was placed in the caudoventral abdomen in the area of the teats. The urinary bladder could be seen only when it was filled with urine.

Conclusions: Transcutaneous abdominal ultrasonography enables a safe technique for the examination of different abdominal organs in SACs. The animals well tolerated the procedure. The use of alcohol as contact medium between probe and skin and the abdication of clipping the hair ruled out to be a suitable technique in SACs and therefore can be recommended. The results of this study show that ultrasonography can be expected to be of great practical diagnostic value in patients suffering from abdominal diseases.

Camelids

P02-002-214

Dexmedetomidine and tiletamine-zolazepam anesthesia in alpacas

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Objectives: To evaluate the effect of intramuscularly administered tiletamine-zolazepam (TZ) with either dexmedetomidine or saline, on immobility, selected cardiorespiratory variables, and recovery quality in alpacas.

Materials and Methods: Five adult, male alpacas were studied using a Latin square design. Each alpaca was given TZ (2 mg/kg) combined with either dexmedetomidine at 5 µg/kg (D5), 10 µg/kg (D10), 15 µg/kg (D15), or 20 µg/kg (D20), or saline (C), intramuscularly at 1-week intervals. The doses of TZ and dexmedetomidine were prepared separately, combined in a syringe, and injected into a semitendinosus or semimembranosus muscle. Immobility, defined as the lack of motor response to claw clamping, was assessed, and characteristics of anesthesia, recovery, and selected cardiorespiratory variables were recorded.

Results: Duration of immobility was longest for animals in treatment D15 (30.9±5.9 minutes) and D20 (40.8±5.9 minutes). Duration of lateral recumbency was significantly longer when animals were given dexmedetomidine. The longest time (81.3±10.4 minutes) to standing was observed in treatment D20. Four control animals moved in response to claw clamping at the five minute time point. Heart rate decreased in all dexmedetomidine treatment groups in comparison with the baseline values. The PaO₂ values for treatments D10, D15, and D20 were significantly less than the control treatment during the first 15 minutes (P<0.05). Three alpacas in the control treatment and one in D5 exhibited muscle stiffness during recovery and had multiple efforts to regain the sternal position; all other recoveries were graded as excellent.

Conclusions: Administration of TZ (2mg/kg) IM without the addition of dexmedetomidine allowed all alpacas to be placed in lateral recumbency, but only one alpaca exhibited a lack of motor response to claw clamping. Dexmedetomidine at 5 µg/kg with TZ (2 mg/kg), induced only a brief period of immobilization; however, doses of 10, 15 and 20 µg/kg increased the duration of immobility.

Camelids

P02-002-215

Establishment of biochemical and haematological reference values for alpacas (Lama pacos) reared in Europe

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Objectives: Small camelids are increasingly encountered in European veterinarian practices. The laboratory tests represent an important tool in the diagnosis of alpaca's diseases. Haematological and biochemical reference values can be influenced by climate, geographical areas or

nutrition. However, all data available were obtained from alpacas reared in America, and no data were available regarding alpacas reared in European conditions.

The aims of this study were to establish reference intervals for biochemical and haematological tests in alpacas, to compare results of automated and manual haematocrit, and to describe normal morphology of blood cells.

Materials and Methods: Blood was collected from clinically healthy female and male adult alpacas (Lama pacos) from the only herd close enough from the veterinary school and its laboratory to ensure proper conservation of samples before analyses.

Blood samples (n = 44) underwent full cell blood count analysis using the Sysmex XT 2000iv analyser. Microhaematocrit measures were also performed manually, using a microhaematocrit centrifuge (centrifugation for 5 minutes at 11000 rpm). Blood smears, stained with May Grunwald Giemsa (MGG) and brilliant Cresyl blue (BCB) staining, were checked for manual differential WBC counts, platelet estimates, and morphologic examination of blood cells.

Blood samples (n=43) were assessed for urea nitrogen, creatinine, and glucose concentrations, and for aspartate transferase (AST), creatine kinase (CK) and γ-glutamyl-transferase (GGT) activities using Vitalab Selectra XL analyser (Vital Scientific).

Reference intervals were established using robust and parametric methods after identification of outlier values and transformation of data that didn't have a normal distribution into a normal one by using a Box-Cox transformation.

Results: Biochemical reference intervals for alpacas reared in France were quite similar to those reported previously on alpacas reared in America, except for higher CK-related results and, to a lesser extent, for AST.

Haematological reference intervals for alpacas, using automated analyser, were also similar to those reported previously, except for lower platelets-related and haematocrit-related results. Correlation between automated and manual microhaematocrit was weak, with a systematic undervaluation of haematocrit when performing automated test in comparison with manual microhaematocrit.

Blood smears results were also consistent with previous description of alpaca's blood cells morphology. However, no Cabot rings were observed. Moreover, small basophilic dots were encountered under the membrane of red blood cells, both with MGG and BCB staining.

Conclusions: This is the first European work published concerning biochemical and haematological reference values for alpacas. Our results will be useful guides for interpreting test results ; however, more herds need to be involved in a second part of this work in order to limit herd bias. Moreover, haematocrit values obtained with automated test should be interpreted cautiously, and manual microhaematocrit should be performed systematically.

Finally, as far as we know, this is the first description of basophilic punctuations in alpaca's red blood cells with MGG and BCB staining; further studies are needed to explain the origin of these punctuations.

Camelids

P02-002-216

High prevalence of "Candidatus Mycoplasma haemolamae" in Austrian llamas and alpacas

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Objectives: Candidatus *Mycoplasma haemolamae* (CMHl) infection in llamas and alpacas may either lead to acute diseases with severe anaemia or chronic diseases associated with emaciation or fertility disorders. However, many animals are rather asymptotically infected playing an important role as pathogen reservoir. Reports of CMHl infections of camelids in Europe are rare and only available from the United Kingdom and Switzerland. Therefore it was the aim of this study to assess the prevalence of CMHl in Austrian llamas and alpacas in order to determine potential risk factors associated with CMHl infection.

Materials and Methods: A representative number of llamas and alpacas were investigated nationwide. The animals included in this study were clinically healthy. Regarding the age of the animals, the llamas and alpacas were divided into two groups, whereupon one group included animals between 1 and 2 years of age and one group included all animals older than 2 years. Blood samples were collected from the external jugular vein using K2 EDTA sample tubes. The blood was examined for CMHl using PCR. Prevalence data were assessed and compared to geographical region, age, sex, species, and origin of the animal using the Chi² test.

Results: From a total number of 461 blood samples examined, collected from 193 llamas and 268 alpacas, CMHl was detected in 119 (25.8 %) samples employing species-specific PCR. Furthermore, CMHl was detected significantly more often in alpacas (*Vicugna pacos*) than in llamas (*Lama glama*) with a p-value of 0.001, and more frequently in younger animals (age < 2 years, p = 0.008). Additionally, regional differences in the prevalence of CMHl have been observed, representing a less frequent infection rate in the western parts of Austria. Although not significant, male animals were infected more frequently. Looking at the origin of the animal no statistically significant difference between animals born in Austria and animals imported from foreign countries was evident.

Conclusions: In the present study a high prevalence of CMHl in apparently healthy animals was observed confirming the important role of healthy animals as pathogen reservoir. Results of this study also indicate furthermore different risk levels of infection between llamas and alpacas and between younger and older animals. Regional differences in the prevalence of CMHl may be associated with climatic differences and/or variations in the presence of appropriate insect vectors. However, the data presented in this study underline the necessity of further studies on the significance of CMHl in llamas and alpacas.

Camelids

P02-002-217

The effect of breeding season on the gestation length of alpacas in the UK

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Objectives: The long gestation period of the alpaca (*Vicugna pacos*) makes continually achieving one offspring per year difficult. There is considerable variation around the average of 345 days. The reasons for this, and possible opportunities to influence it, are poorly understood.

Outside their native South America, alpacas are non-seasonal breeders. In the UK, most breeders choose to mate between Easter and early autumn. In horses, gestation length differs depending on breeding season. Similar findings were reported for alpacas in New Zealand. This study aimed to establish whether this phenomenon could be observed in the Northern Hemisphere.

Materials and Methods: Reproductive data spanning one to eight years was collected from five alpaca farms in the South East of the United Kingdom (latitude 51°32'N). Most animals were of the Huacaya type. All farms had a similar breeding regime, using pen mating at weekly intervals until the dam became non-receptive. Mating dates and parturition dates were obtained. Data from two farms also allowed calculation of birthing rate (defined as number of dams producing a live cria out of all dams mated). Pregnancy was assumed to have resulted from the last recorded mating for each dam.

Matings before or on the 21st June of each year were classed as 'spring', and those after 21st June as 'autumn'. The summer solstice was chosen based on extrapolation from seasonal-breeding species that are influenced by daylight length.

Unpaired T-tests were used for statistical analysis of gestation lengths, assuming equal variances. Fisher exact test was used for birthing rates.

Results: The gestation length for 736 dams was available. Average gestation length was 345.4 days (SD 13.0 days). Spring matings resulted in a gestation length that was 10.9 days longer than autumn matings (p<0.0001). This pattern was found for all farms in the study. From May to September, for every day mating occurred later gestation length decreased by 0.1 or 0.3 days, respectively, depending on the type of trend line fitted.

The birthing rate was significantly lower in autumn (74.0%) compared to spring (82.7%; n=639; p<0.02), and more matings were performed per dam in autumn.

Conclusions: Breeding season does affect the gestation length of alpacas in the UK, and autumn matings would appear to be beneficial to increase reproduction rates. However, the lower birthing rate and higher number of matings in autumn may negate this advantage.

Similarly, there may be a difference in survival and growth rates of crias born in either spring or autumn. Studies on these and other aspects are required before advice can be given on the optimal mating season to maximise reproductive performance in UK alpaca herds.

Further studies are required to elucidate what causes the difference in gestation length.

Camelids

P02-002-218

Blood serum steroid, thyroid hormone, and insulin concentrations in healthy male and female alpacas

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Objectives: Although native to South America, alpacas have gained popularity in several countries including the United States. With this increase in popularity comes the need for veterinary care including the determination of species specific reference intervals to aid in evaluating health status. Therefore, the objective of this study was to determine the concentration of adrenal and gonadal steroids, thyroid hormones, and insulin in male and female alpacas.

Materials and Methods: Physically healthy male and female Huacaya and Suri alpacas from privately owned farms in Tennessee were used in this study. Blood samples were collected from the jugular vein of 37 males (35 intact, 2 castrated; age 1-15 years) and 53 intact females (age 2-12 years) from August to October, 2015. Blood serum was stored at -80°C until analyzed for cortisol, progesterone, 17-hydroxyprogesterone, estradiol, androstenedione, testosterone, thyroxine (T4), triiodothyronine (T3), and insulin at The University of Tennessee Clinical Endocrinology Service.

Results: Hormone results (min-max) for male and female alpacas, respectively, were <1.0-2.9 µg/dL and <1.0-1.5 µg/dL (cortisol); <0.20-0.36 ng/ml and <0.20-5.36 ng/ml (progesterone); 0.06-1.04 ng/ml and 0.02-0.24 ng/ml (17-hydroxyprogesterone), <10.0-36.8 pg/ml and <10.0-44.7 pg/ml (estradiol); 0.03-2.05 ng/ml and 0.04-0.93 ng/ml (androstenedione); 19.4-403.0 ng/dL and <15.0 ng/dL (testosterone); 4.2-11.8 µg/dL and 3.9-10.3 µg/dL (T4); 48.7-403.0 ng/dL and 72.4-380.0 ng/dL (T3). Insulin concentration was <2.0 µIU/ml for all alpacas in the study except for 1 male (4.4 µIU/ml) and 1 female (2.5 µIU/ml), and testosterone concentration in the castrated males was <15.0 ng/dL. For cortisol, progesterone, estradiol, and testosterone, several animals were below (as indicated by the < symbol) the lowest standard of each hormone assay. Approximately 80% of female alpacas in this study have progesterone <0.20 ng/ml. At this time, it is not known if the female alpacas with progesterone above <0.20 ng/ml are pregnant or if these values reflect luteal activity without pregnancy.

Conclusions: The hormone concentrations determined in this study provide an initial endocrine database for male and female alpacas. This information aids in physiological characterization of alpacas and can be used by veterinarians in the clinical evaluation of suspected endocrine dysfunction.

Camelids

P02-002-219

Successful protocol of caesarian section in Arabian camels (*Camelus dromedaries*) in the Emirate of Abu Dhabi, UAE

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Objectives: Seventy eight caesarean operations from different breeds of camels were performed during the period between October 2013 and April 2015, Abu Dhabi Emirate. All cases were diagnosed as dystocia and were unsuccessfully managed by mutations or fetotomy. The causes of dystocia were recorded. The present study was designed to describe successful protocol of caesarian section in camels under field condition in the Emirate of Abu Dhabi, with references to indications, preoperative preparation, operative site, anesthesia, surgical technique, post operative care, operative and post-operative complications and dam and calf survival.

Materials and Methods: Seventy eight caesarean section operations of different breeds of camels (Sudani, Omani, Hazmi and Crossbred, 10, 44, 11 and 13 animals, respectively) were performed. These caesarean sections were done under field conditions by a veterinary surgeon specialist at Al-Tiba Veterinary Hospital, Abu Dhabi Emirate, during the period between October 2013 and April 2015. All cases in

this study were diagnosed by the veterinarians as dystocia and were unsuccessfully managed by mutations or fetotomy. The inspection data, camel and farm identification and the cause of dystocia were recorded. Information pertaining to the sick camel examined during the study was collected. This includes identity of camel, housing and management, concurrent disease, body weight, age, duration of dystocia, previous dystocia history, feed intake and medication of camel. Seventy two camels presented at the time of operation have poor health status and six camel cases are under shock condition, since they had severe dystocia due to emphysematous (4 cases) and macerated (2 cases) fetus. A new procedure of caesarian section has been applied in Arabian camels. This included preoperative preparation, operative site, restrains and anesthesia, surgical technique, post operative care, operative and post-operative complications of dam. Data were analyzed using Freq procedure of SAS (2004). Frequency and percentages were calculated and tested by chi square test.

Results: Out of seventy eight outpatient camels under caesarian section, the overall percentage of dam survival was 92.31% (72/78), with significant differences among different breeds (P=0.0286). The highest percentage was in Hazmi and Crossbred (100.00%, each respectively) followed by Omani 93.18% and Sudani breed 70.00% (Table. 1). Observations with regard to the percentage of fetal survival after caesarian section were also recorded (Table 1). The percentage of fetal survival was 28.21% (22/78) with no significant differences among breeds (P=0.3390). Data summarized in Table 2 indicated a highly significant differences (P=0.0001) in the fetal survival between the different age groups, while no significant (P=0.2796) differences in the dam survival was found between the age groups. Post operative complications are presented in Table 3. Two suture types (chromic catgut usp 3 and Safil no. 3) were used for caesarean section in the present study. Infected wounds (33.33%) and abdominal hernia (22.22%) were the main postoperative complications. Numerically, Safil no. 3 was more effective suture used in the caesarian sections than chromic catgut usp 3 (P: 0.4075 and 0.079, for infected wounds and abdominal hernia, respectively). The percentage of wound infection (36.73% and 27.59%) and abdominal hernia (28.89% and 11.11%) used chromic catgut usp 3 and Safil no. 3, respectively.

Conclusions: Left ventro-lateral oblique incision is the most preferable approach for caesarian section in camels. Safil no. 3 was more effective suture used in the caesarian sections than chromic catgut usp 3. No significant difference on dam survival was found using the two suture types. The successful outcomes of caesarian section in camels in our study suggested that good health of the dam, adequate pre and post operative preparation, safety anesthesia, and perfect surgical technique are the most important key elements that redound to minimize the detrimental postoperative complications and high dam survival.

Camelids

P02-002-220

Dose recommendations for effective treatment of endoparasitism in South American Camelids

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Objectives: Dicrocoeliosis and nematodiasis are major health concerns in South American Camelids farmed in Europe. Prevalence of resistance was reported against benzimidazoles and macrocyclic lactones. Albendazole which can be used to treat dicrocoeliosis in other species has been reported to be toxic in camelids. In llamas and alpacas, drugs are used in an off-label manner, since no products are licensed for these species. For most drugs no studies on dosing are available. A novel oral formulation enabled a study on dosing praziquantel in llamas infected with *Dicrocoelium dendriticum* and monepantel was evaluated to treat gastrointestinal nematodiasis.

Materials and Methods: Study procedures were discussed and approved by the institutional ethics committee of the University of Veterinary Medicine Vienna in accordance with Good Scientific Practice Guidelines and national legislation.

53 llamas of mixed sex were included in the praziquantel study (S1) and 46 llamas in the monepantel study (S2). Faecal examinations confirmed natural infection with the respective parasite of interest. Animals were weighed and randomly allocated to one of the treatment groups or the control group. Animals of treatment groups in S1 were dosed orally using a praziquantel-containing paste specifically produced for high-dose treatment (250 mg/ml) at a dose of either 25 mg/kg BW PO (group 1) or 50 mg/kg BW PO (group 2). Monepantel (Zolvix, Novartis Healthcare, Denmark) was dosed orally in S2 at the dose rate recommended for sheep (2.5 mg/kg BW, group 1), 5 mg/kg BW (group 2), or 7.5 mg/kg BW (group 3). Untreated control groups were used for monitoring natural changes in egg counts during the studies. Faecal samples were collected directly from the rectum of each animal before and 14 days after treatment and examined on the same day or stored at 4°C for processing next day. The employees performing faecal examinations were not aware of the treatment status of the respective animals. Criteria for efficacy were faecal egg count reduction (FECR %) and extensity effect (EE %). The FEC was determined by using a modified McMaster technique with a lower threshold of 25 eggs per gram.

Results: In S1 the mean BW (\pm SD) of animals of groups 1 and 2 were 122.6 \pm 27.7 kg and 113.6 \pm 25.9 kg, respectively. Llamas of the control group had a mean BW of 104.6 \pm 32.8 kg. In S2 the mean BW (\pm SD) of animals of groups 1, 2 and 3 were 110.4 \pm 43.9, 120.1 \pm 31.7 kg and 113.6 \pm 25.9 kg BW, respectively. Llamas of the control group had a mean BW of 109.4 \pm 41.9 kg.

None of the animals (S1, S2) showed adverse reactions to the treatment including changes in appetite, attitude, physical activity, urination or defecation as reported by the owners.

Praziquantel treatment in S1 led to a mean FECR of 85% in animals of group 1 with an EE of 67% whereas therapy in group 2 led to a mean FECR of 91% with an EE of 81% implying that almost twice the number of animals of group 1 still shed eggs two weeks after treatment compared with group 2.

Monepantel treatment in S2 led to a mean FECR of 84% with an EE of only 25% in group 1. Mean FECR obtained from animals in group 2 was 93% with an EE of 67%. Animals of group 3 were all tested negative for gastrointestinal nematodes two weeks after treatment (FECR: 100%, EE: 100%).

Conclusions: Results of S1 indicate that 50 mg/kg BW oral praziquantel is required for efficacious dosing against *Dicrocoelium dendriticum* in SAC. The novel drug formulation using a high concentration of praziquantel in a small volume of palatable paste is suitable for applying the required high amount of praziquantel safely.

The results of S2 show that monepantel is highly effective when administered at 7.5 mg/kg BW. The dose rate recommended for sheep (2.5 mg/kg BW) did not lead to effective results. Even at a dose rate of 5 mg/kg BW, monepantel turned out to be underdosed. Thus monepantel is recommended at a dose rate of 7.5mg/kg BW in SAC.

Camelids

P02-002-221

Intraocular melanoma in a 10-year-old female llama

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Objectives: The objective of the presented study is the description of the surgical treatment of an intraocular melanoma in a 10-year-old female llama with a continually growing mass associated with the left eye.

Materials and Methods: A female llama was presented to the Clinic for Ruminants with a continually growing mass associated with the left eye, which had first been observed five months previously. The llama's general condition was good; the left eye showed severe exophthalmus. The cornea was completely covered by an ulcerating mass which obstructed the view on deeper ocular structures. The lens could not be seen at all. An ophthalmological examination was performed to exclude the possibilities of a foreign body in the eye, a fungal infection, intraocular cysts or a parasitic disease such as nematodiasis. The suspected diagnosis was an intraocular tumor. As the llama's general condition was good without indication of a spreading of the disease when doing a thorough clinical examination, enucleation of the left eye was considered the best surgical treatment. This operation was performed under general anaesthesia.

Results: Enucleation is a technically easy operation, enabling surgeons to reduce painful processes within the area of the eye and to stop the progression of neoplastic structures. The operation was performed without the occurrence of any complications. After the enucleation, histological examination was carried out. The mass was characterized as an amelanotic melanoma. The Antibiotic and analgesic therapy was continued after the operation for four days, and the patient was discharged to go home 10 days after the enucleation. As these tumors are known to lead to spreading to the regional lymphatic nodes, especially in humans and dogs, a clinical examination was performed seven months later. The llama was in good health and showed no problems in the herd because of the unilateral blindness. An inquiry by telephone 14 months later revealed that the llama is still in good health.

Conclusions: The most common reason for eye diseases in camelids is trauma, but ocular diseases caused by tumors have been increasingly reported in these species. Intraocular tumors can be classified as either melanocytomas (non-malignant) or melanomas (malignant). As the whole eye was affected without any physiological structures left, the possibility of a non-perforating keratectomy was not considered, but an enucleation of the affected eye was performed. The mass was characterized as an amelanotic melanoma. There are only a few publications on melanomas in new world camelids, but spreading metastases of the tumor are also possible in these species.

Comments: The literature lies with the author.

Camelids

P02-002-222

Camel Toxoplasmosis: Seroprevalence and Zoonotic aspects in Cholistan desert Pakistan

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Objectives: Toxoplasmosis is a parasitic zoonosis with worldwide distribution caused by *Toxoplasma gondii* and is demonstrated in domestic livestock, wild life, other animals and humans. Camel milk and meat is consumed in Pakistan and Cholistan desert area is considered as a hub and native place for the camels. The present study was carried out to determine the prevalence and zoonotic potential of camel toxoplasmosis in Cholistan desert area, Bahawalpur.

Materials and Methods: Serum samples from 226 camels and 226 humans (n=113 camel keepers; n=113 humans with no contact history to camels) were collected and analyzed for anti-toxoplasma-antibodies. Data about each camel and human were entered in a "data capture form". The serum samples collected from camels and humans were processed for anti-toxoplasma antibodies by using Latex Agglutination Test (LAT) at dilutions 1:16, 1:64, 1:128 and 1:256. Finally percentage prevalence was calculated and analysed statistically by Chi square test.

Results: Of the camels tested, 9.73% were seropositive for anti-toxoplasma antibodies at titers of 1:16 or higher. As to gender, 11.1% female camels and 8.69% male camels were positive for anti-toxoplasma antibodies. The highest prevalence (12%) was observed in camels of 11 year or above age group followed in order by 6-10 year (9.8%) and 1-5 year (4.4%). The prevalence of toxoplasmosis was high in the 3rd trimester of pregnancy (18.1%) while in the 1st and 2nd trimester it was 10.5% and 13.3%, respectively. Overall, 12% humans were seropositive for anti-toxoplasma antibodies. The highest seroprevalence of toxoplasmosis was observed in camel keepers (15.92%) than people with no contact history to camels (7.96%). As to gender, 11.97% women and 11.76% men were seropositive for anti-toxoplasma antibodies. Age related prevalence of anti-toxoplasma antibodies was 15.25% in persons between 31-40 years or above age followed in order by 12.8% in 21-30 years, 9.61% in 15-20 years and 5.88% in ≤15 year old age groups.

Conclusions: It was concluded that toxoplasmosis is prevalent among camels in Cholistan desert area of Bahawalpur, Pakistan and their is high risk of transmission of toxoplasmosis to humans having contact with these camels.

Materials and Methods: A 3-year old female alpaca, 10 months in gestation, was presented with the complaint of anorexia since 3 days, and a suspicion of a uterine torsion. Rectal ultrasound revealed no abnormalities in the uterus. The animal aborted shortly after admission. Clinical examination, blood examination, ultrasound, radiography and computed tomography (CT) was used in the diagnostic work-up of this case.

Clinical examination revealed muffled heart sounds and dyspnea, but no abnormal sounds on auscultation of the lungs. Hematology, blood gas analysis and ionogram were normal, as were kidney- and liver values. Triglycerids in blood were 0.26 mmol/L (reference <0.11 mmol/L). Ultrasound of the thorax revealed a large amount of anechogenic fluid in the thorax. At the ventral aspect of the lung surface, comet-tail artifacts were noticed, indicative for abnormal lung tissue. Under ultrasound guidance the fluid was punctured. The obtained liquid was white with a bluish aspect, and contained a total protein of 40g/l, triglycerids of 1.72 mmol/L, and no cells.

Results: Radiography revealed a higher opacity of the caudoventral lung parts and an alveolar pattern. CT-images indicated a collapse of the left cranial lung lobe, and a remarkable fluid-opacity in the ventral aspect of the thorax, with local foci of a clear alveolar pattern. On both sides of the thorax, there were several pleural noduli. In the right hemithorax, a large space-occupying structure with a size of 12cm in length, 1 cm in width and 2 cm in height was visible.

The macroscopic aspect and high triglyceride in the pleural effusion confirmed chylothorax in this patient. The large structure and different noduli visible on the CT-image suggested the presence of a neoplastic process, although a granulomatous pathology could not be excluded. Granulomas can be caused by chronic bacterial, fungal or viral infections, by aspiration of foreign bodies or by immune mediated reactions.

Two liters of fluid were drained from the thorax using thoracocentesis. Control-ultrasounds revealed no further accumulation of fluid after initial draining. The animal was treated during 1 months with penicillin to treat a potential chronic bacterial pneumonia. General condition gradually improved, and the animal fully recovered. No signs of relapse occurred in a one year telephonic follow-up period.

Conclusions: The authors did not find any previous cases of chylothorax in alpacas in peer-reviewed literature. There were no indications for a traumatic cause in the anamnesis. Abortion only occurred after diagnosis of the fluid in the thorax. In this particular case, the chylothorax was apparently caused by compression of the ductus thoracicus by a mass of unknown nature. The positive evolution after antimicrobial treatment suggests a chronic bacterial pneumonia as the primary cause of this granulomatous swelling in the lung and consecutive chylothorax.

Camelids

P02-002-223

Chylothorax in an alpaca caused by a granulomatous swelling in the lung

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Objectives: A chylothorax (or chyle leak) is a type of pleural effusion. It results from lymph formed in the digestive system called chyle accumulating in the pleural cavity due to either disruption or obstruction of the thoracic duct.

The objective of the present study was to describe a case of chylothorax in an alpaca.

Clinical Genetics

P02-002-224

Genetic Background Of Tibial Hemimelia Syndrome In Galloway Cattle

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Objectives: Tibial hemimelia syndrome (TH) has been reported in single cases in Galloway, Shorthorn or Bunaji cattle. Affected calves are usually stillborn and exhibit multiple anomalies including shortened or absent tibia, agenesis of the patella, abdominal hernia, cryptorchidism, failed Müllerian duct development, hirsutism, meningoencephalocele, and internal hydrocephalus. We hypothesized that the *Aristaless-Like Homeobox 4 Gene (ALX4)* is a candidate gene for TH in Galloway cattle due to its key role in limb development and the identification of causative mutations within this gene in TH cases in humans, mice and Shorthorn cattle.

Materials and Methods: Two Black Galloway calves affected by TH were born in springtime 2014 in a herd of a part-time farmer in Saxony/Germany. Pathological and microbiological investigations, X-ray analysis and maceration of the hind limbs were performed. Tissue samples of the TH affected Galloway calves and 13 hair or blood samples of their relatives were provided for genetic studies by the breeder.

The bovine *ALX4* gene is located on BTA15q28-q29 between positions 74,452,084–74,486,658 Mb and harbours 4 exons coding for a protein of 397 amino acids. For comparison of sequences, exons and adjacent intronic regions were amplified using PCR. PCR products were purified and sequenced using standard protocols. For sequence comparison and identification of sequence variations seven animals of the cattle family including the two affected calves were used. Raw DNA sequence data were manually checked for ambiguities using NC_007313 (Btau 4.6.1) and AC_000172 (UMD3.1) as reference sequence. For in silico prediction of splice site mutation effects NNSPLICE 0.9 and Human Splicing Finder were used.

Furthermore, fluorescence resonance energy transfer (FRET) assays were developed for high throughput genotyping of the detected duplications in exon 2 and 4 of *ALX4*. A total of 1688 DNA samples of the most common Galloway varieties (Black/Red/Belted/Riggitt Galloway: GA), and 289 White Galloway (WGA) as well as 876 German Holstein Friesian (HF) and 86 DNA samples of 21 different cattle breeds were randomly selected from the DNA depository of the Institute of Veterinary Medicine Göttingen/Germany and investigated.

Results: Both TH affected Galloway calves were stillborn and showed multiple malformations, i.e. abdominal hernia, arthrogryposis multiplex, and syndromic acerebral macrocephaly. An X-ray analysis and maceration of the hind legs of one calf revealed a specific aplasia of the tibia and the absence of the pubic bone. Infectious causes for the malformation were excluded by negative microbiological and serological analysis of common protozoa, bacteria and viruses in the dams and calves.

From the pedigree analysis an autosomal recessive inheritance of the defect can be predicted. DNA sequence comparison of the coding and flanking intronic regions of the *ALX4* gene revealed 10 SNPs in seven animals of the Galloway cattle family. The SNPs in the coding regions were synonymous mutations. In exon 2 and exon 4 duplications of 20 bp and 34 bp were detected in the affected animals and their parents. Both duplications result in a frameshift leading to a premature stop codon and

truncated protein. The affected calves were homozygous only for the duplication in exon 2. Both cattle were paternal half-siblings. The sire was heterozygous for the duplication in exon 2. The dam of case 1 was carrying both duplications and the mother of case 2 was heterozygous only for the exon 2 duplication.

The duplications were only detected in Galloway cattle. In GA the exon 2 duplication allele frequency was 0.01 and the exon 4 duplication allele frequency was 0.23. WGA showed higher frequencies for both duplications with 0.06 and 0.38, respectively.

Conclusions: In Shorthorn cattle, a DNA-based test for TH is available based on the US patent US 8,158,356 B2, claiming a large deletion of more than 129 kb of BTA15q (75.18–75.31 Mb) including exon 1 of the bovine *ALX4* gene. Since this deletion differs from the mutation identified here, the test is not applicable in Galloway cattle. We identified a duplication of 20 bp in exon 2 of the *ALX4* gene resulting in a frameshift and disruption of helix III of the homeodomain as causative mutation for TH in Galloway cattle. Now it will be possible to test the population using the established FRET assay and implement genotyping results into breeding programs.

Complementary Veterinary Medicine

P03-003-063

Cerebrospinal fluid analysis of 30 ruminants with central nervous system diseases

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Objectives: Ruminants may be affected by a wide variety of central nervous system (CNS) diseases, including bacterial, viral and protozoal diseases; toxic and metabolic disorders, trauma or neoplasms. This paper aimed to report the cerebrospinal fluid (CSF) results from 30 ruminants diagnosed with CNS diseases.

Materials and Methods: The medical records of ruminants with CSF analysis of registration were evaluated. The study included thirteen sheep, twelve cows and five goats, totaling 30 ruminants. The cases were included only when the diagnosis was confirmed by the association of epidemiological, clinical, laboratory and histopathological data. In suspected cases of enzootic ataxia and spinal cord trauma, therapeutic diagnosis through supplementation of copper and positive response to the therapy with anti-inflammatory and esteroideais thiamine, confirmed the clinical suspicion, respectively. CSF samples were obtained by puncture of the cerebellomedullary cistern (n=26) or at the lumbosacral site (n=4) after surgical preparation.

Results: In the 30 ruminants examined, the conclusive diagnosis consisted of botulism (8 cases, 26.7%) polioencephalomalacia (5 cases, 16.7%), conidiobolomycosis (3 cases, 10%), suppurative meningitis (3 cases, 10%), pituitary abscesses (3 cases, 10%), spinal cord trauma (3 cases, 10%), meningoencephalitis (2 cases, 6.6%), and single cases of thalamus abscess, myelitis and enzootic ataxia (1 each case, 3.3%). Neutrophilic pleocytosis (20-997 leukocytes/ μ L), presence of fibrin clots and turbid aspect were seen in cases of bacterial infection of the CNS, such as meningoencephalitis, meningitis, cerebral abscesses, myelitis and a case of conidiobolomycosis (fungal infection). In two cases of medullar trauma, CSF analysis showed xanthochromia and presence of phagocytosed red blood cells. All cases of polioencephalomalacia, enzootic ataxia, botulism and two cases of conidiobolomycosis showed no CSF alterations (2-9 leukocytes/ μ L). A case of cervical abscess causing pathological fracture between C5 and T1 vertebrae revealed no changes in CSF analysis, probably because the abscess did not infiltrate to the *dura mater*.

Conclusions: Authors reiterate that evaluation of CSF is a valuable diagnostic tool in association with epidemiological and clinical data for diagnose of CNS diseases in ruminants. The infectious component remains as a important cause of CNS disease in ruminants from Northeastern Brazil.

Complementary Veterinary Medicine

P03-003-064

Biomedical Treatment With The Extract Of Mangosteen To Promote Beneficial Microbiota And Increase Voluntary Feed Intake Of Lactating Calves.

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Objectives: It is objective was observed in group two effects of mangosteen extract and xanthone 9-xanthene providing greater nutrient intake, important in the physical development of calves when they enter the stage productive area. The perspective with the mangosteen extract and xanthone 9-xanthene pretend stimulated activity against Enterobacteria of the gastrointestinal tract that promotes the intestinal health of nursing calves.

Materials and Methods: Herbal extract the aqueous ethanol solvent, whose components and / or fractions obtained from the mangosteen plant, with antibacterial ability was selected for its effect in vitro inhibition of bacterial group; enterobacteria face in the gastrointestinal tract (GIT) of the animals. The extract of mangosteen (*Garcinia mangostana* Lynn) have antibacterial properties against infections of the respiratory and digestive tract. In this paper the antibacterial activity in vitro was observed for these selected bacteria, the value of the minimum inhibitory concentration (MIC) of mangosteen extract and its fractions; supernatant and precipitate precipitate dehydrated. The farnesiana nanocellulose from Acacia XG® commercial product, 9-xanthene® xanthone and between these and the mixture ranged biomedicamentosos compounds 25, 50 and 100% concentration and 5 to 10 mg / mL respectively. In this work the effect of mangosteen extract administered orally in calves of Holstein-Friesian breed in the infancy period was also observed. Control calves fed milk replacer kalbermilch-premium (trow balance®) group, called three treatments were used. Group 2 calves fed mangosteen extract 25% and 5 mg of xanthone 9-xanthene® in milk replacer, and Group 3 with calves fed 50% mangosteen extract. Weight gain, height calves and quantification of colony forming units (CFU) of pathogenic and non-pathogenic bacteria in infancy: the variables were recorded

Results: The results showed that in the halos of bacterial inhibition for enterobacteria significant difference (P <0.05); The highest value (mm) was in the XG® commercial product (20 ± 0.6) followed by the mangosteen extract (16 ± 1.15) xanthone 9-xanthene® (7 ± 0.73) and nanocellulose (1 ± 0.12). The results showed that consumption of food concentrate weight per day in group 2 (40.2 ± 0.1 kg) was significant (P <0.05) than that recorded in the control group (32 ± 2.3g), but similar to group 3 (36.2 ± 0.1 kg). Consumption of milk replacer in aqueous solution exists significant difference (P <0.05) in the control group (166 ± 3.5 L versus 152 ± 1.2 ± 2.9 L and 145 L) for group 2 and 3, respectively. The daily weight gain of calves (883 ± 88.2g, 600 ± 572 ± 57.7g 36.5g), no significant difference (P <0.05) between treatments was recorded; Group 2, Group 3 and control, respectively. At the height increase was not significant (P <0.05), it is recorded (89.7 ± 2.0 cm day⁻¹, 85.7 ± 3.7 cm day⁻¹ and 85 ± 0.6 cm day⁻¹) for group 2, the control group and group 3, respectively. Same no significant effect (P <0.05) in the final chest circumference (100.3 ± 2.3 cm day⁻¹, 95.7 ± 1.3 cm day⁻¹ and 93.7 ± 0.9 cm day⁻¹), group 2, group 3 and group control, respectively. In bacterial cultures there was significant difference in UFC mesophilic bacteria (P <0.005). The highest value was in group 3 (4639 ± 1425), followed by 2 group (3279 ± 1312) and the control group (1961 ± 684). What coliforms face identified in animal feces (CFU) were recorded in percentage.

Conclusions: It is concluded that the greatest achievement in the consumption of concentrate was observed in group 2 effects of mangosteen extract and xanthone 9-xanthene® providing greater nutrient intake, important in the physical development of calves when they enter the stage productive. Also with mangosteen extract the xanthone 9-xanthene® stimulates activity against enterobacteria of the gastrointestinal tract that promotes intestinal health of nursing calves

Complementary Veterinary Medicine

P03-003-065

Comparison of Radioimmunoassay and VIDAS methods for progesterone and estradiol determination in dairy cow serum.

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Objectives: Radioimmunoassay method (RIA) is the most important method for measurement of steroid hormones levels. It is easy, cheap, and sensitive but needs special equipment and laboratories to perform because of using radioactive agents. Evaluation of P4 and E2 using VIDAS method is based on enzyme-linked fluorescent assay (ELFA). This method is very easy, rapid, safe, comparable in price with RIA (but equipment is much cheaper and easier to calibrate) and it could be used in field conditions. The aim of this study was to compare RIA and VIDAS methods for P4 and E2 bovine serum concentrations.

Materials and Methods: The study was performed on 38 dairy Holstein-Friesian cows and heifers ranged from 18 months to 9 years of age. All animals included to the study did not show any signs of clinical diseases. Eight animals were pregnant (1 heifer, 7 cows) in different stages of gestation (ranged from 49 days to 6 months). Additionally 6 of them showed oestrus behaviour during pregnancy without any disturbances of gestation.

The blood samples were collected from jugular vein in 10 ml syringe and immediately put to the test-tube. After transport to the laboratory within 2 hours all samples were centrifuged and serum was stored at -80 °C until evaluation was performed (within 4 weeks).

Progesterone and estradiol concentrations in every sample were evaluated using two methods: enzyme-linked fluorescent assay (VIDAS) and radioimmunoassay method (Wizard) to compare results.

Statistical Analysis

The Shapiro-Wilk test was performed to determine whether the data was normally distributed. The non-parametric Wilcoxon matched pairs test was used to assess differences between estrogen and progesterone level results obtained by RIA and VIDAS methods. A Bland-Altman [Bland and Altman 1986] assessment for agreement was calculated to compare the two methods. A range of agreement was defined as mean bias $\pm 2SD$. Correlations were evaluated using the Spearman test. A value of $p < 0.05$ was chosen as the limit for statistical significance.

Results: Mean estrogen concentrations obtained by RIA and VIDAS methods were 5.47 ± 5.20 pg/ml and 12.99 ± 9.47 pg/ml, respectively. Paired Wilcoxon test confirmed significant differences between the two methods in estrogen level results ($p < 0.0001$). Mean progesterone concentration obtained by RIA was also significantly different compared to results obtained by VIDAS ($p < 0.0001$) (table 1). Despite a significant difference between the progesterone level results determined using the two methods, there was a close correlation between the two ($r = 0.973$, $p < 0.0001$) (figure 1).

To further examine whether there was an agreement between hormone level measurement methods, the Bland and Altman [1986] plot of the mean versus the difference of the values obtained from these methods was performed.

Conclusions: Differences between estradiol and progesterone concentrations received in ELFA and RIA methods respectively were statistically significant, so we conclude that enzyme-linked fluorescent

assay is no equivalent to RIA method and it couldn't be considered as reference method.

Complementary Veterinary Medicine

P03-003-066

Proteinogram And Serum Biochemistry Of Holstein Cows In The Peripartum Period

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Objectives: The aim of the present study was to evaluate the kinetics of proteinogram, enzymatic activities and levels of minerals and lipids in blood samples obtained from primiparous and multiparous Holstein cows in the peripartum period.

Materials and Methods: Blood samples were obtained from 24 primiparous and 24 multiparous Holstein cows by jugular venipuncture using vacuum tubes without anticoagulant in three occasions: 60 days (M60), and 30 days (M30) pre-partum and immediately after parturition (M0). Blood samples were centrifuged at $1,000 \times g$ for 10 minutes, and serum samples were stored at -20 °C until analysis. Electrophoretic separation of serum proteins was carried out in polyacrylamide gels containing sodium dodecyl-sulphate (SDS-PAGE) and biochemical analysis of blood serum was performed by spectrophotometry using commercial reagents. Data obtained were analyzed by ANOVA and differences between groups or moments were compared using Tukey's test, considered significant when $P < 0.05$.

Results: Serum concentrations of total protein, albumin, globulins, magnesium, cholesterol, as well as gamma-glutamyl transferase activity were higher in multiparous cows, while serum levels of ceruloplasmin, total calcium and alkaline phosphatase activity were higher in primiparous cows. The moments influenced concentrations of total protein, globulins, immunoglobulin A, immunoglobulin G and transferrin, which were lower in the day of parturition as compared to the other sampling times by 17%, 25%, 43%, 73%, and 25.5%, respectively, in both groups. Concentrations of ceruloplasmin, and haptoglobin increased an average of 54.4% and 28.9%, respectively, in both groups, also in moment 0. Multiparous cows, when compared to primiparous cows, showed a more pronounced decrease in levels of total calcium (13.8% versus 7.2%), phosphorus (10.9% versus 9.88%), cholesterol (34.8% versus 18.4%) and triglycerides (39.5% versus 30.6%) in the day of parturition.

Conclusions: Based on these findings, it can be concluded that the influence of parity should be taken into consideration when evaluating acute phase protein concentrations, enzymatic activities and mineral and lipid concentrations of high yielding transition cows.

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Diagnostic Imaging

P02-002-055

Tibial nerve paresis in post partum German Holstein cows - Etiology and Treatment under farm conditions

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Objectives: Peripheral nerve injuries are the most frequent bovine neurological disorder. Further the tibial nerve paresis is the most frequent cause of lameness in milking cows after parturition in modern husbandry systems beside claw diseases. This abnormality in movement is shown in a hyperflexion of the hock and a forward knuckling of the fetlock joint. The aim of the study is to develop a diagnostic and therapeutical procedure and verify these under farm conditions.

Materials and Methods: In a period from July 2013 to January 2016 in 50 cows the diagnosis of a tibial nerve paresis was made. In 28/50 cases the cows were presented on farm visits and in 22/50 cases the cows were stationary patients in the Clinic for Ruminants and Swine in Leipzig. In 20/50 cases no treatment were made, because in twelve cases the owner didn't want any treatment, in six cases the symptoms were present only for 24 hours and in two cases the cows had to be euthanized in consequence of more severe diseases (coma hepaticum, bilateral pyelonephritis). The other 30 cows were treated in the following way. After initial systemic treatment with antibiotics and steroidal antiphlogistics (each cow received an intravascular injection of 0.4 mg/kg BW dexamethasone) the affected leg was stabilized from the coronary band to the distal aspect of the tarsal joint using a cast made of synthetic resin. The cast was removed after 28 days by splitting it laterally and medially into two parts. These two parts were brought together as a new bandage remained for two more weeks. Then a new supporting bandage for more two weeks was applied in the same dimension. In 14/29 cases this treatment was performed completely in the farms and 16/29 were initially treated in the clinic. In nine of the 16 cases the cows were intensively monitored and controlled based on the project design. This included a stationary stay for one to two weeks, daily clinical examination, laboratory diagnostics and partially the sonographic and electrophysiological examination of the hindlimbs.

Results: In 8/30 cases there was too much pressure on the limb from the cast which resulted in decubitus wounds, mostly presented in severe cases of tibial nerve paresis with a high grade of hyperflexion and knuckling. By changing the treatment schedule in form of removing the cast earlier and treating for a longer time with supporting bandages the paresis could be treated in seven cases. In one case the complications were too severe so the cow was brought to the slaughter house. In each of the 30 cases there were no more signs of a tibial nerve paresis after treatment (usually after 56 days, most often after 28 days).

Conclusions: The short regeneration time in each case shows that a low grade damage of the nerve is present. If there is a severe damage of the nerve with following WALLERIAN-degeneration the regeneration would need a time of two month to one year, depending on the localization of the damage. This study presents an way to treat the tibial nerve paresis in case of a lowgrade nerve damage. There are a lot of reasons for the damage of the tibial nerve in post parturient cows. With increasing the collection of data concerning the preliminary reports in following studies it will be possible to find out the cause and prevent tibial nerve paresis.

Diagnostic Imaging

P02-002-056

Ultrasonographic Anatomy of the Bovine Temporomandibular Joint

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Objectives: Reports of temporomandibular joint (TMJ) disorders in cattle are rare. The low incidence may possibly be explained by the difficulty in diagnosing TMJ disease. Clinical symptoms are not specific, feed intake and mastication are impaired, and opening of the mouth is painful. Clinical findings may include swelling, pain, and fluctuation. The TMJ is difficult to physically investigate in cattle presenting with a clinical problem in this area.

Therefore the objectives of this study were to report the normal ultrasonographic appearance of the TMJ in cattle, and to delineate sonopathological findings of the TMJ assessed in two patients.

Materials and Methods: A 7.5 MHz linear and a 6.0 MHz convex transducer (Mindray™ DP 50 Vet, Mindray Medical International Ltd., Shenzhen, China) were used for the TMJ examination in twelve healthy Holstein-Friesian cows with a mean age of 68.9 months, in four cadavers and in two cattle showing clinical signs of TMJ disease. Ultrasonography was carried out in standing cattle with the animals restrained in a crush. Each TMJ (n: 24) was scanned in a rostralateral (RL), lateral (L) and the caudolateral (CL) plane. The distance from the surface of the skin to the zygomatic process of the temporal bone and the distance from the skin to the lateral edge of the condylar process (CP) of the mandible were measured. In addition, the echogenicity and characteristics of the bone surfaces, TMJ space, joint capsule, disc, muscles, adjoining blood vessels and the parotid salivary gland were described.

Ultrasonographic images were compared with the findings of gross anatomical dissection and frozen anatomical sections of this region in four cadavers.

Results: The lateral canthus of the eye, the base of the ear, and the visible zygomatic arch in between served as landmarks for palpation of the TMJ region. The transducer was then placed perpendicularly mid-way between the lateral canthus of the eye and the base of the ear over the zygomatic arch, resulting in the L-plane. The ventral tip of the transducer then remained over the CP of the mandible, while the dorsal tip of the transducer was rotated 45° rostrally to obtain the RL-plane or 45° caudally resulting in the CL-plane. The relevant joint-forming bone surfaces, blood vessels, muscles and the parotid salivary gland could be imaged in all normal TMJ. Using the linear transducer the articular disc could never be visualised, and the joint capsule only in 70.8% via the CL-plane. Using the convex transducer, the TMJ capsule could be imaged in 100% of joints in the CL-plane, and in 54% of cases the lateral parts of the disc could be depicted. It was never possible to differentiate the TMJ pouch in healthy cattle.

In contrary, in two bovine patients a marked anechoic and heterogeneous hypoechoic effusion of the TMJ with distension of the joint capsule could be depicted.

Conclusions: This study describes the ultrasonographic appearance of the TMJ region in healthy cattle for the first time. These results provide reference for ultrasonography of pathologic conditions of the TMJ region in cattle. Since other diagnostic imaging techniques, such as CT or MRI, are not feasible for the majority of bovine patients, the authors would recommend that all bovine veterinarians, who are equipped with at least 7.5 MHz linear rectal probes, should use ultrasonography for further investigation of clinical cases with masticatory problems of unclear origin to exclude or diagnose TMJ disorders.

Diagnostic Imaging

P02-002-057

Ultrasonographic Estimation Of Forward Or Backward Rotation Of The Distal Phalanx Within Cow's Claws

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Objectives: Ultrasonography has been previously applied for measuring sole thickness, and the distance between the sole surface and basal surface of the distal phalanx (S-D distance). However, forward or backward rotation of the distal phalanx has not yet been evaluated quantitatively in previous applications of ultrasonography to the claws. The present report included methodological evaluations to quantitatively estimate forward or backward rotation of the distal phalanx using ultrasonography, to clarify correlations with computed tomographic (CT) measurements (Tsuka et al., J Dairy Sci 2014;97:6271-6285).

Materials and Methods: 267 pairs of hind limbs, which were obtained from lactating Holstein cows in a slaughter-house, were used to be examined using CT (Pronto SE or ECLOS; Hitachi, Tokyo, Japan). 150 of 267 pairs of hind limbs were used to be examined using ultrasonographic machine (HI VISION Preirus; Hitachi-Aloka Medical, Tokyo, Japan: measurement accuracy was 0.1 mm). Slight trimming was applied to all claws before CT and ultrasonographic examinations for purpose of making flat, smooth sole surfaces by improving irregularities, and maintaining original hoof shapes. Sole thickness and S-D distance were measured at 2 points on mid-sagittal 2D CT and ultrasonographic images: point A was located on the virtual line from the most apical margin of the distal phalanx to the sole surface; and point B was located on the virtual line from the most caudal margin of the distal phalanx to the sole surface. Data at point A were subtracted from data at point B (B-A sub). The relative angle of the distal phalanx to the sole surface (S-D angle) was calculated from the angle between the virtual line along the apex to the flexor tuberosity in the distal phalanx and sole surface. Estimation accuracies were investigated using Pearson correlation coefficients between S-D angle and B-A sub of the sole thickness, and between S-D angle and B-A sub of the S-D distance on CT and ultrasonographic images. Correlations between B-A subs of S-D distances and S-D angles on CT and ultrasonographic images were investigated using linear regression analysis. A p value of <0.05 was considered statistically significant.

Results: In applications of ultrasonography to the 600 claws of 150 pairs of hindlimbs, the B-A subs of sole thickness, and S-D distance could be evaluated in 531 claws (88.5 %), and 551 claws (91.8 %), respectively. The Pearson correlation coefficient between S-D angles and B-A subs of sole thickness was significantly high at $r = 0.743$ ($p < 0.05$), and $r = 0.582$ ($p < 0.05$), but tended to be lower than those between S-D angles and B-A subs of S-D distances ($r = 0.783$, and $r = 0.684$; $p < 0.05$) in CT and ultrasonographic measurements, respectively. The linear equations describing the relationship between B-A subs of S-D distance and S-D angles were $y = 0.62x + 2.77$ in ultrasonographic measurements, and $y = 0.83x + 2.15$ in CT measurements, where y represents the S-D angle and x is the B-A sub of the S-D distance obtained from ultrasonographic images.

Conclusions: According to the linear equation describing the relationship between B-A subs of S-D distance obtained from ultrasonographic images and S-D angles, the optimal S-D angle of 3.0° (Tsuka et al.,

2014) is likely acquired by hoof trimming as the S-D distance in the region of the heel becomes approximately 0.4 mm thicker than that in the region of the toe. On the other hand, a critical S-D angle of 6.0° (Tsuka et al., 2014) is related to an S-D distance at the heel >5.2 mm thicker than at the toe on ultrasonographic images.

Comments: Rotations of the distal phalanx forwards or backwards to the sole surface inside the bovine claw are well known as the causative factors of sole horn disorders. However, it has not yet been evaluated quantitatively in previous applications of ultrasonography to the claws, although ultrasonography has been useful for measuring sole thickness, and the distance between the sole surface and basal surface of the distal phalanx. The present study indicates that the rotations of the distal phalanx can be estimated by measuring sole thickness, or the distance between the sole surface and basal surface of the distal phalanx in 2 points of the sole surface. For making the linear equations, a great many specimens have been examined using CT and ultrasonography. We believe that the present data will be very reliable, and very useful for field applications of ultrasonography to bovine claws.

Diagnostic Imaging

P02-002-058

Investigation to local complications after the retrograde intravenous regional anesthesia of the toe of dairy cattle

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Objectives: The retrograde intravenous regional anesthesia (RIVA) is a common technique on the distal bovine limb. There are reports about different complications from clinics in the literature. Aim of the study was to prove the innocuousness of the RIVA under field conditions.

Materials and Methods: The investigation was realized in a freestall barn on 44 German Holstein cows (age: 4.8 ± 1.5 years, bodyweight: 610 ± 73 kg, milk yield: 43 ± 10 kg/day). The cows suffered superficial to perforating lesions of the pododerma (N = 33) on one hind limb or were healthy animals (N = 11). After the shave, cleaning, defatting and disinfection of the injection area we performed a RIVA on one hind limb of every cow (plantar common digital vein IV, N = 37; dorsal common digital vein III, N = 7). The tourniquet leaved 25 min in every case. A bandage below the dewclaws was applied. The injection area was checked clinically and with ultrasonography one, four, six and 24 days after the RIVA.

Results: One day after the injection we detected superficial skin irritations (N = 3) and an increase of the skin thickness without (N = 2) and with acute inflammation signs (N = 1) in the injection area. At that time we found edema of the hypoderm on seven animals during the ultrasonographic examination. Four of these animals were also clinical conspicuous (skin irritation N = 1; increased skin thickness N = 3). Overall we detected nine cows with alterations in the injection area. Six of these animals had a pododermatitis and three were healthy cows. In each case of these animals the injection was performed in the plantar common digital vein IV. Hematoma or thromboses were not diagnosed. All superficial skin irritations recovered three and the increased skin thickness in the injection area not later than six days after their first appearance.

Conclusions: The skin irritations are obviously small irritations in connection with the shave of the injection area. Aspect for that is the location of these lesions in the lateral injection area, which was not protected from the bandage. The edema of the hypoderm could be a sign of a local dermatitis or a partial paravenous application of the local anesthetics. We could not find a negative effect for the healing of the claw dermis lesions. The located complications were mild and recovered without any further therapy. In our opinion the RIVA is an excellent and save anesthesia for the veterinarian practice in the farms.

Diagnostic Imaging

P02-002-059

Methodology for mammary thermography in dairy cows

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Objectives: The objective of this experiment were determine best methodology for conducting the mammary thermography, in environment of production, in 123 crossbreed dairy cows, that are in lactation peak and high milk production.

Materials and Methods: Animals with problems in clinical examination, other than mastitis were treated and exclude from the study, to avoid ambiguous infrared thermography data.

Thermography pictures were made with the Flyer® thermographer, Thermal resolution is automatically calibrated to ambient temperature.

The pictures was performed 0.5 meters away from the udder, lateral images for fore quarters and caudal and side images for rear quarters. The pictures always followed the same sequence that began right fore quarter, right rear quarters, left rear quarter and finally left fore quarter. The caudal projection described by Pezenshke et al. (2010) was performed to compare with the lateral projection to determine the best field projection for rear quarters.

To avoid mastitis influence on thermographic temperatures, mammary quarters were categorized: No changes (0 milk score in strip cup and SCC (somatic cells count) less than 200 × 10³ cells); b) subclinical mastitis (0 milk score in strip cup and SCC greater than 200 × 10³ cells); c) clinical mastitis (milk score 1 or greater in strip cup).

For evaluation mammary thermography, minimum and maximum temperatures of most uniform area of quarter were tabulated, as described by Schmidt (2008), Hovinen et al. (2008), Polat et al. (2010) Pezenshke et al. (2010). Data was subjected to analysis in the statistical program SAS (2003) and IBM SPSS Statistics 19. Comparison of mean temperatures obtained according to the group (no changes, subclinical mastitis and clinical mastitis) was performed by PROC MIXED option REML

Results: When comparing separately maximum and mean of fore quarters with rear quarters, rear quarters was higher (p <0.05), independent of quarter SCC. It's going against reported by Schmidt (2008) who described fore quarters with higher temperature (0.6 ° C) than rear. It probably did not occur in this study due data use from

lateral projection (minimum, maximum and mean temperatures) for the evaluation of rear quarters, which are protected by medial surface of hind limbs, preventing heat loss to environment. However, when evaluating mammary thermography of rear quarters by caudal projection and fore quarters by lateral projection (DIFTOT), the thermal temperature was similar between each categories (p > 0.05) in different times. Likewise, Poikalainen et al. (2008) report a similar (p > 0.05) between the temperature of the udder surface between fore and rear quarters right or left when working with dairy cows.

However, when evaluating DIFTOT or DIFCENT that using the side projection for rear and fore quarters observed similarity (p > 0, 05) between fore and rear quarters, regardless of subclinical or clinical mastitis.

Conclusions: The temperature difference between maximum and minimum enable correction any distortion caused by mammary quarter position and external factors. Because it will increase the minimum and maximum temperature. In this study there was no statistical difference between use of caudal projection for rear quarters and side projection in fore quarters. The recommendation is use caudal view for evaluation of rear quarters to be agile, easy, practical and better suited to production system.

Diagnostic Imaging

P02-002-060

A new endoscopic approach for bronchoalveolar lavage - compared with traditional transtracheal lavage for bovine respiratory disease diagnostics

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Objectives: Bronchoalveolar lavage (BAL) is a very common and useful diagnostic approach for bovine respiratory disease (BRD) management. Traditionally the lavage is carried out through the trachea (trans tracheal lavage – TTL). A new hand held multiscope (endoscope with several different functions; Ivetscope, Quidee, Germany) has been designed for a more convenient way of BAL under field conditions. The aim of this study was to compare two different methods of BAL – the puncture of the trachea with the transtracheal lavage against the oro-laryngeal method.

Materials and Methods: The new endoscope approach was applied on 64 calves (age 2 – 22 weeks) with bovine respiratory disease (BRD) history. Bronchoalveolar liquid was sampled for microbial and virological evaluation. 32 calves were collected by the traditional way (TTL), whereas 32 calves were examined by the use of this new endoscope (BAL). The pistol-shaped, hand-held and cordless endoscope, reaches 40 cm in length, with 2 working tunnels. A camera is positioned next to the corpus, so that a visual adspection can be carried out simultaneously. Calves were sedated (xylazine, 10mg/kg) prior to treatment, kept into sterno-ventral position with the head held manually by the farmer. Each time two samples of broncho-alveolar liquid were collected: From the deeper part of the lung (>50 cm distance) and from the upper part of the bronchus (30-40 cm). They were split equally into a modified New York City medium (NYC medium; Biocheck) and a sterile vessel for further examination.

Results: On average in a total of 15,4% of all cases no agent could be identified. With this new method only in 6,1% (4 times less!) of all cases the responsible microbiological cause could not be identified, whereas

the traditional technique reveals 25% "no agents" ($p < 0.01$).

Single strains of bacteria/virus results were obtained two times more often with the BAL method (60.6% vs. 34.4%) than with the TTL approach.

BRSV was found in total of 9.2% of all analysed samples, with no difference between the two methods.

M. bovis was found three times more often (33.3% vs. 9.4%) with the endoscope lavage (BAL).

P. multocida was the most single prominent bacteria to be found in the lung, in total 9.2%. 15.6 % of all cases monocausal cases are diagnosed with *P. multocida* by TAL vs. 3.0 % by BAL; whereas BAL revealed *P. multocida/M. bovis* 5 times more often than with the TAL method (15.2% vs. 3.1%).

Conclusions: The new BAL method with the Ivetscope seems to result in an obviously more precise identification of relevant respiratory tract bacteria.

M. bovis might be more difficult to collect in traditional TTL and could be overgrown by other bacteria as the mixed infection and contamination rate is significantly higher in TTL sampling.

As the Ivetscope method is also less invasive and more rapid than the TTL method, it offers a more reliable and practical way for BRD diagnostics and herd screenings.

Diagnostic Imaging

P02-002-061

Assessment of Broad Ligament ultrasonography as a novel technique for endometritis diagnosis in dairy cows.

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Objectives: The objective of this study was to investigate any possible relationship between the anatomical characteristics of the Broad Ligament, as shown by ultrasound images obtained from dairy cows after calving, and the incidence of endometritis in these same cows. Since ultrasonography is already used by many farm practitioners during cow fertility examinations, extending its use to the diagnosis of endometritis would increase the value of this medium with no extra capital investment for the veterinarian or need for further sampling and laboratory analysis.

Materials and Methods: dairy cows were recruited from six dairy farms, cows and calved heifers that had been calved 9 - 87 days were recruited.

Using a manual vaginal scoop, any vaginal discharge present was noted and graded on the recognised 0-3 scale (Sheldon and Dobson 2004).

A cross-sectional view of the previously pregnant uterine horn 2-4 centimetres anterior to the uterine body was visualised, captured and stored, using a bovine B-mode ultrasound scanner with an 8.5 MHz linear array probe. Similarly, an image of the ipsilateral Broad Ligament ventral to this point was obtained and stored. The ultrasound probe was adjusted to give the greatest degree of Broad Ligament vascular cross-sectional area in each image at the time of examination to produce as consistent a measurement as possible between animals.

The proportion of fluid in the Broad Ligament was determined by delineating the boundaries of the Broad Ligament and setting contrast threshold limits to select the areas within the Broad Ligament that had

a pixel contrast consistent with the echogenicity of fluid. The fluid area was then expressed as a percentage of the area within the boundaries of the Broad Ligament. The three largest vessels in the images of the Broad Ligament were selected and the diameter of each was measured.

Results: The proportion of fluid in the Broad Ligament and the diameters of the three largest lymphatic vessels (vessels 1, 2 and 3) were greater in animals with endometritis compared to controls ($P < 0.001$, $R^2 = 0.34$, 0.22, 0.21 and 0.19 respectively). The mean (\pm SEM) proportion of fluid in the Broad Ligament was $32.0 \pm 1.7\%$ in cows with endometritis and $18.8 \pm 1.2\%$ in cows without endometritis. The mean (\pm SEM) diameters of the three largest vessels in the Broad Ligament of healthy cows were 2.5 ± 0.2 mm, 2.0 ± 0.1 mm, 1.7 ± 0.1 mm but in cows with endometritis the vessels were 1.4 ± 0.3 mm, 0.9 ± 0.1 mm and 0.8 ± 0.2 mm wider respectively.

Conclusions: The Broad Ligament is a novel focus for ultrasonography and specific changes within the images from this structure are indicative of the presence of endometritis. Changes in the fluid content and vascular dimensions can be measured and are a highly significant indicator of the presence of endometritis. Cows with endometritis have almost twice as much Broad Ligament fluid and wider lymphatic vessels than cows without endometritis. Broad Ligament ultrasonography is a cost-effective, non-invasive tool that gives rapid results in the diagnosis of uterine disease and may be able to help determine the location of the infection or inflammation.

Diagnostic Imaging

P02-002-062

Venography In Distal Digits After Intraruminal Oligofructose Administration

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Objectives: This study proposed to describe venographic alterations in the pelvic digits of the crossbred (*Bos indicus* X *Bos taurus*) steers after laminitis induced by intraruminal administration of oligofructose.

Materials and Methods: For this study were used seven crossbred (*Bos indicus* X *Bos taurus*) non castrated steers aging 12.6 ± 0.9 months and weighting 156 ± 37.8 kg. The animals were sorted in two groups (GI and GII) receiving 13 and 17 g/kg, respectively, of the oligofructose trough the rumen. Venography of pelvic distal limbs were done in different moments, 36 hours (M1), seven (M2), 15 (M3) and 30 (M4) days after intraruminal administration of oligofructose. With the venography it was intended to evaluate possible changes involving main veins and those observed in coronary corium and bulbar regions of bovine pelvic digits, having M0 as control. The diameters of common digital dorsal vein III, plantar branch to digital cushion and dorsal distal phalanx vein were measured and the values were compared by T test at 5% of significance. The main veins and those from coronary corium, at the different moments, were evaluated descriptively.

Results: Changes in vascularization at different moments were identified, like vasodilation with 36 hours of oligofructose administration and vasoconstriction at other times, in the main veins and at coronary corium and bulbar net. On animals of higher dose of oligofructose changes were more prominent. There were statistical differences for distal dorsal digital vein III between M1 and M4 moments in each group. For GI, the normal diameter of this vein was 0.387 ± 0.023 cm, in M1 was 0.479 ± 0.103 cm and in M4 0.378 ± 0.063 cm. For GII, the

normal diameter for distal dorsal digital vein III was 0.406 ± 0.084 cm, in M1 0.445 ± 0.085 cm and in M4 0.341 ± 0.029 cm. Therefore, there was vasodilation in M1 and vasoconstriction in M4. To the plantar branch of digital cushion of medial digit there was difference between groups at M4, with more vasoconstriction in GII (0.087 ± 0.079 cm) comparing to GI (0.195 ± 0.032 cm).

Conclusions: It could be concluded that venographic examination of the pelvic digits of the crossbred (*Bos indicus* vs *Bos Taurus*) steers, after intraruminal administration of oligofructose, permits the description of the local vascular anatomy and the identification of changes compatible to laminitis.

Diagnostic Imaging

P02-002-063

Biometry Eye Measurements by Ultrasonography in Jersey Breed Cattle: Preliminary Study

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Objectives: The aim of this study was determining the normal measurements of the eye chambers of the bovine Jersey breed and describe the features of the eyes, by B-mode ultrasonography, to provide normal references values.

Materials and Methods: The ocular biometry was done in 40 healthy Jersey breed cattle aging between 3-36 months. The young animals were contained manually and adults in the chute. The head should be restrained by two assistants to facilitate the centralization of ocular bulb and minimize any sudden movements. Ultrasound examination of each animal was performed using linear transducer selected in 12 MHz (GE Logic Healthcare®) after topical anesthetic eye drop administration. The sterile acoustic gel was used and the ultrasonographic examination was performed without pressing the transducer against the ocular surface. A single investigator executed all ultrasound examinations. The measurements in the right and left eyes were taken in vertical axial section and horizontal axial section. In the both sections the axial length (AL - distance from the epithelial layer of the cornea to the posterior wall of the eyeocular bulball), the anterior chamber depth (AC - from the Descemet's membrane to the anterior lenticular capsule), the lens thickness (LT - distance between the anterior to the posterior lenticular capsule) and the vitreous chamber depth (VC - distance between the posterior lenticular capsule to the back wall of the ocular fundus) were measured. The means and standard deviation of measurements for the right and left eyes were presented. The means were compared by t test with 5% level of significance.

Results: The cornea was identified as two parallel but convex hyperechoic lines, separated by a thin anechoic line. The lens appeared as two hyperechoic lines, and a convex (anterior capsule) and a concave (posterior capsule) separated by anechoic content. Adjacent to the anterior lens capsule was observed the iris and the ciliary body, both with hypoechoic appearances. The ciliary body is thicker and irregular when compared to other domestic species. The vitreous chamber and the anterior and posterior chambers appeared as an anechoic region. The back wall of the eye was seen as a regular and concave hyperechoic line. Means and standard deviations of the AL, AC, LT and VC observed in the horizontal axial section, to the left vs right eyes, were as follows: (3.07 ± 0.17 vs 3.08 ± 0.17 , $P = 0.99$), (0.49 ± 0.04 vs 0.50 ± 0.04 , $P = 0.87$), (0.98 ± 0.07 vs 0.98 ± 0.07 , $P = 0.99$) and (1.53 ± 0.08 vs $1.54 \pm$

0.07 , $P = 0.74$). For vertical axial section, were found, respectively: (3.07 ± 0.18 vs 3.08 ± 0.18 , $P = 0.97$), (0.49 ± 0.05 vs 0.49 ± 0.05 , $P = 0.92$), (0.98 ± 0.07 vs 0.98 ± 0.07 , $P = 0.82$) and (1.53 ± 0.07 vs 1.54 ± 0.07 , $P = 0.99$).

Conclusions: The dimensions of the right and left eyes are equivalent by ultrasonography biometry. The mode-B ultrasonography is a quick, minimally invasive and safe method which resulted in important information about the intraocular dimensions, making it possible to standardize the eye measurements of specific breeds in different age groups. These results could be useful for diagnosis of diseases that change the size of the chambers of the eye.

Diagnostic Imaging

P02-002-064

Validation of an easy-to-use device for non esterified fatty acids (NEFA) assay in blood in dairy cattle

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Objectives: The presence of non esterified fatty acids (NEFA) in blood is an indicator of lipolysis and are thus interesting markers of negative energy balance of post partum dairy cows. NEFA seem more reliable indicators than beta hydroxybutyrate, widely assayed by vet practitioners thanks to the availability of portable cow-side devices. The main limiting factor for NEFA assay in practice is the requirement of an appropriately equipped laboratory. The aim of this study was thus to validate NEFA assay results obtained through a spectrophotometer in field conditions, i.e; of easy use at the veterinary clinic.

Materials and Methods: Blood (5 ml) was collected on dry tubes from the coccygeal vein from 2 weeks before and 2 weeks after calving in 100 cows from 6 herds. Blood was stored at -4°C until centrifugation, and serum separated into two aliquots frozen at -20°C until analysis.

One aliquot was assayed by spectrophotometry at the veterinary clinic (Vetphotometer Diaglobal GmbH, Berlin, Germany) following the manufacturer's instructions. Measurement range indicated by the manufacturer goes from 0.02 to 4 mmol/L.

The second aliquot was analysed by automated colorimetry (RANDOX kit, London, UK), considered as the reference method. Measurement range goes from 0.04 to 2 mmol/L. Intra and inter assay coefficients of variation are below 5%.

Correlation between the results obtained by the two methods was evaluated by a Spearman test with $p < 0.05$ considered as statistically significant.

Results: Intra and inter assay coefficients of variation for the tested spectrophotometry method were respectively 4.3% and 9.6%. Correlation between values obtained by the spectrometer and the reference method is high, with a Spearman's coefficient of 0.96 ($p < 0.001$). Both values were linked by the following equation: $[\text{NEFA}]_{\text{photometer}} = 0.581 [\text{NEFA}]_{\text{reference}} - 0.0018$. The commonly used threshold for NEFA blood concentration defining negative energy balance by the reference method is 0.3 mmol/L (Ospina et al 2010; Chapinal et al, 2011) to 0.5 mmol/L (Leblanc et al 2005) when assayed 2-14 days before calving. After

calving, the threshold value changes to 0.72 mmol/L with the reference method (Ospina et al 2010). These thresholds for the spectrophotometer can be calculated thanks to the equation to respectively 0.17 to 0.29 mmol/l before calving and 0.41 mmol/l after calving.

Conclusions: Measuring NEFA concentration in blood of dairy cows with the spectrophotometer is reliable, simple, quick (results in 20 minutes) method and does not require any specific equipment (except the photometer). The spectrophotometer is thus an appropriate tool in practice for energy balance monitoring of dairy cows during the transition period.

Diagnostic Imaging

P02-002-065

The use of thermography to measure ocular temperature and its correlation with rectal temperature in cattle

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Objectives: This study aimed to correlate the temperature of the ocular region recorded by the infrared camera and rectal temperature measured using digital thermometer in healthy calves. Infrared thermography is a modern method, non-invasive and safe to perform a thermal profile. With wide application in human medicine for diagnostic purposes, in veterinary medicine its use is still restricted. In cattle, a key advantage is it does not require direct physical contact with the animal, which would facilitate and expedite the diagnosis of various diseases

Materials and Methods: Thirteen holstein calves, healthy and up to thirty days old were used. The animals were housed within the Cattle Hospital and Small Ruminants FMVZ - USP, in covered and out of direct incidence of sunlight and wind location, taking care to achieve a reliable infrared thermography image, avoiding dirt and moisture in the animal's hair coats, considering the effect of circadian rhythm (the data were collected at the same time), the time after eating, exercise and pre-existing skin lesions. Through a thermographic camera, with colorful palette, images of ocular region was collected and concomitant rectal temperature was measured using a digital thermometer. During the analysis the animals were maintained on station. Eighty-nine data collection were performed and subsequently analyzed by a specific software. Thermal information of each pixel has been processed thus generating the maximum temperature point of the particular area. Therefore, an orbital image was obtained by drawing an oval image including the eyeball. The mean, standard deviation and Pearson correlation coefficient was verified.

Results: Based on the ratio of the results of rectal temperature and temperature of the ocular region, were obtained for the rectal temperature the mean and standard deviation of 39.09 ± 0.53 °C and the temperature of the ocular region obtained through the thermograph of 38.67 ± 0.72 °C. For the Pearson correlation coefficient (r) the value found was 0.58, making it possible to affirm that it is positive and medium. The temperature of the eye region obtained by the thermal imager was an average of 0.42 °C lower than the rectal temperature.

Conclusions: Although the infrared thermography camera is good accuracy tool for determining the temperature of any object in addition to the convenience of its use, enabling the verifying changes in temperature

without direct contact with the animal in this study proved not to be of great value in the accurate check of the parameter in question. Indeed, there needs to be more research and development of standard operating procedures for the use of thermographic camera for this technique to be used reliably.

Diagnostic Imaging

P02-002-066

Use of infrared thermography for the observation of blood vessels at the outer ear of calves

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Objectives: The thermographic examination is conceptualized as the image obtained through an infrared camera, which detects accurate temperature differences, by means of color variations in a thermogram. Through the assumption that the temperature detected by the camera on the skin surface is related to the blood flow and local metabolism, the aim of this study was to collect pictures of calves' outer ears using a thermal camera (FLIR T620) with a black and white output in order to identify the blood vessels of the region.

Materials and Methods: Through a thermal camera (FLIR T620) with black and white output, images were taken at the outer ear of five black and white Holstein healthy calves. The animals were housed at the Cattle and Small Ruminants Hospital - FMVZ in a covered area, without direct incidence of sunlight and weather. All images were carefully taken to achieve a reliable thermal image, avoiding dirt and moisture at the skin, considering circadian rhythm's effects, if animals had been fed or exercised, the application of topical medications and pre existing scars or lesions.

Results: With images taken with the thermal camera (FLIR T620) at the outer ear is possible to visualize, in a grey scale, the vessels conformation of auricular region in contrast to the cartilage. The blood vessels were visualized in a whitish color, corresponding to a higher temperature area, while the external ear cartilage was visualized in a grayish color, corresponding to a lower temperature area. It can also be seen at the vessels images that there is color homogeneity, demonstrating its integrity and suggesting a fully functional organ.

Conclusions: The study demonstrates that thermography is a reliable method to visualize the blood vessels of the outer ear of calves, as well as its integrity and any possible vascular changes. Moreover, it is a noninvasive method, which allows the examination at a short distance from the animal, which is very important when dealing with cattle. The use of thermal imaging is relatively new in veterinary medicine, however it has shown very good results and it could be widely used as a possible diagnostic method and in assistance to veterinary practice.

Diagnostic Imaging

P02-002-067

Sonographic evaluation of the involution of umbilical cord components in Holstein calves during the first month of life

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Objectives: The purpose of this study was to evaluate the physiological involution of umbilical components of healthy calves by means of ultrasonography, considering the use of 2% or 5% iodine tincture, used in navel treatment during the first days after birth.

Materials and Methods: Evaluation was performed by ultrasonography of intra and extra-abdominal umbilical components in nine different positions, distributed between the xiphoid cartilage and pelvis, following the paths of the intra-abdominal umbilical components. They were characterized regarding the appearance of the images, the measures of the diameter and thickness of vascular structures and urachus, as well as the peculiarities from the two types of disinfecting. Twenty-three Holstein male calves, reared in dairy property located in the State of Sao Paulo, Brazil, were evaluated from birth to 30 days old and for the examinations, the animals were kept in right lateral decubitus.

Results: Commonly found in calves, diseases of the umbilical components can lead to serious complications. Diagnosis of umbilical diseases is often not fully enlightened by bimanual abdominal palpation, since this semiotic method is not sensitive enough to determine the extent of involvement of the intra-abdominal umbilical structures in part of the cases. Thus, ultrasonography is a valuable complementary test for establishing an accurate diagnosis on the location and extent of intra-abdominal omphalitis. Our results showed that the vein and umbilical arteries lose their blood vessels characteristics, assuming a ligament aspect due to fibrous tissue proliferation. This process followed a pattern, in which the fibrous tissue was initially present in the inner part of the vessel wall, heading toward the center of the vessel lumen as the involution evolved. This process of involution was earlier in the parts of the blood vessels that were farthest from the external navel, with no particular differences with the use of different antiseptic concentrations. Measurements of the diameter of the umbilical components and the thickness of their walls were also standardized during their physiological involution. We also observed that, before the decrease of these measures, there was an increase in the diameter of these structures and the thickness of their walls. As these changes occurred between the 15th and the 20th days after birth, ultrasound observations confirmed that this period corresponds to the time when the vessels are losing their features and turning into a ligament.

Conclusions: Our results confirm the accuracy of ultrasonography for the assessment of intra-abdominal umbilical components during the first 30 days of life the calf and establish references to improve the diagnosis and the choice of treatment of umbilical diseases.

Diagnostic Imaging

P02-002-068

Radiographic examination of Epiphysitis in fattening bulls and corresponding pathological aspects.

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Objectives: Epiphysitis is an inflammation of the growth plate frequently detected in young beef cattle at the level of the distal metaphyses in the metatarsophalangeal joint.

In literature, this pathological process is associated with rapid growing and high weight increase, traumatic injuries, inadequate environmental conditions, toxicosis, excess or deficiency in diet, hereditary or genetic causes.

The early diagnosis of this disease is still not easy and new detection methods must be studied to identify epiphysitis. The purpose of this study is to identify and differentiate the grades of epiphysitis through the use of X-ray images.

Materials and Methods: This study carried out radiological and pathological findings performed on 54 legs in young bulls. Twenty-eight young fattening bulls from 3 different farms were included in the study (10 Aubrac breed; 10 Limousine breed; 8 Charolaise breed). The average age was 19±4 months and the body weight mean values were 480±65 kg. All the animals were bred 8 per box on hard slatted floor.

Animals treated with antibiotic and/or antinflammatory medication were not included.

On clinical examination the animals presented a second degree of lameness, swelling of the metatarsophalangeal joint which was not painful or warm, no limb hypertermia, rapid weight loss, difficulty in keeping the standing position.

X-Ray of the limbs were performed for all the animals, both for the clinically normal and lame. The bulls were sedated with Xilazine 0.25 mg/Kg IM.

The radiographic examination was performed at the level of the distal metaphyses in the metatarsophalangeal joint using a portable X-ray unit AJEX Meditec® (140 H).

The growth plate lesions were measured with an imaging software (Carestream®Image Suite 3.02.0.141).

The total metric survey of the epiphysis (Total S), three metric surveys on the medial part of the epiphysis (S1-M, S2-M, S3-M) and three metric surveys on the lateral part of the epiphysis (S1-L; S2-L; S3-L) were measured in the images considering the sub-axial line of the metatarsus perpendicular to the metatarsophalangeal joint.

Post-mortem pathological analysis were performed for all the animals.

The data collected were statistically analyzed with SAS 9.3.

Results: The radiographic and the pathological findings of 54 young bulls limbs showed lesions on the growth plate and the area of the epiphysis in 44 cases, although 10 limbs did not show any alterations.

The growth plate lesions presented 4 grades of epiphysis inflammation. The radiographic changes in the growth plate thickness were the following:

- Grade 0 (G0): No growth plate lesions (10 limbs);
- Grade 1 (G1): Growth plate slightly broadened (11 limbs);
- Grade 2 (G2): Multifocal lesions thickening of the growth plate (11 limbs);
- Grade 3 (G3): Partial thickening of the growth plate. Partial degeneration of the bone tissue with wide margins,

large and irregular epiphysis (11 limbs);

Grade 4 (G4): Severe thickening of the total area of the growth plate.

Very irregular broad and wide growth plates (11 limbs).

These lesions were confirmed comparing the radiographs with the gross pathological aspects of the lesions.

Statistically significant differences ($P < 0.001$) were found in total metric mean values (\pm St. Dev) survey on every stages of epiphysitis (G0 < 1mm; G1 = 5.9 ± 2.4 mm; G2 = 8.5 ± 1.2 mm; G3 = 11.4 ± 1.05 mm; G4 = 14.7 ± 2.7 mm).

No statistically significant differences were found only for G2 in S1-M versus G1, S2-M, S3M, S1-L versus G3.

Conclusions: In conclusion, our findings on the metric radiographic examination showed that it is possible to distinguish four grades of epiphysitis measuring alterations in the growth plate.

The different grades of epiphysitis had different metric features associated with pathological changes in the growth plate.

The only lesions of epiphysitis indistinguishable in some cases using radiographic metric measures were the G2 from G3. This is due to the presence of multifocal lesions diffuse (G2), not always found in the same location and with variable size in the growth plate.

volume observed in the inspection of the ventral cervical region and the rise of palpable volume in the oropharynx besides the presence of pustular formations and moving deficit epiglottis. In subsequent tests, it was shown that the occurrence of dorsal displacement was intermittent and is not present at all times of the examinations. The conservative treatment of the disease with dexamethasone (20 mg) for three days was successful and was not necessary surgery.

Conclusions: Through the results it was possible to confirm the diagnosis of intermittent dorsal displacement of the soft palate and confirm this case as the first description of this disease in cattle in Brazil and the third case report described in the literature.

Diagnostic Imaging

P02-002-069

Intermittent dorsal displacement of the soft palate in cattle

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Objectives: The objective of this case report is to show that non-routine exams in buiatrics clinic (respiratory system), like endoscopy, can be used to assist the diagnosis of respiratory disease in an adult bovine.

Materials and Methods: An adult cow was referred to the clinic Cattle and Small Ruminants Veterinary Hospital of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo, with history of cough always when ruminated and progressive loss of weight for about two months. Pulmonary auscultation revealed the presence of pathological noises characterized by bilateral dry rales (wheezing), without changing the respiratory tract (costo-abdominal), increased respiratory rate and the presence of thrills compatible with the occurrence of dyspnoea. With use of open mouths internal inspection was conducted to assess possible changes determined by the increase of cervical volume, being observed changes of dimensions in the oropharynx. By direct palpation the region was examined revealing soft consistency in epiglottis, suggesting sagging structure, but without noticeable relative change to its size. After clinical examination and change in finding epiglottis, there was the indication for rinolaringotracheobroncoscopic examination to assess the anterior and posterior airway. For this procedure the animal was restrained and sedated with xylazine (0.2 mg / kg) and anesthetized locally with spray lidocaine in the nasal cavities to pass of the flexible videogastrosopic apparatus (Fujinon EC250LP5) 11.0 millimeters in diameter and 1.60 meters long.

Results: During endoscopic examination, the animal had yellowish mucous and congested blood vessels throughout the length of the airways, swelling in the oropharynx, compatible with the increase in

Education

P01-001-001

The Use Of Photovoice To Assess Livestock Health Needs Of Maasai Pastoralists.

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Objectives: The Ngorongoro Conservation Area (NCA) in Tanzania is the homeland of Maasai. Maasai are pastoralistic livestock keepers who depend on their cattle, goat and sheep as sources of income and food. Pastoral livestock production can be significantly improved by addressing the veterinary needs of livestock owners. We initiated a needs assessment using photovoice. Photovoice leads to an enhanced understanding of community assets and needs, and to empowerment and motivation of the participants. The objective was to use photovoice to (i) build relationships with the NCA community and (ii) strengthen our understanding of their animal health needs.

Materials and Methods: The photovoice methodology was adjusted to the NCA setting; participants used digital cameras, interviews were performed using a laptop to display all the pictures taken by the participant for discussion.

The photovoice method consists of several phases: (i) identification of participants, (ii) pre-interview and training in the use of cameras, photography technique and asking consent of subject, (iii) photography period, (iv) larger or smaller group discussions, and (v) data analysis. Participants gave informed consent for the pre-photovoice interview and debrief separately.

An initial interview was performed to understand the perspectives of Maasai on animal health and their relation to animals as well as to introduce the topics for the photography part of the project. All participants were asked the following questions:

1. What are animals to the Maasai, and why are they important?
2. How are animals important for human health?
3. What is important to keep animals healthy?

Equipped with an automatic digital camera, participants were directed to take pictures that best represented animal health over the next 7 days.

After having discussed all the photos in a debrief, participants were requested to select the 10 pictures that best represented their story. In the final step, the participants were invited to reflect on the photovoice process and make a comparison with the regular interview (pre-photovoice).

Results: The number of photos taken ranged from 28 to 597. All photos were discussed, and only one participant was asked to identify the 40 most important photos. Each recording of the debrief sessions was transcribed and read by two researchers. Key words were identified and clustered in three major themes: animals, human health, and social topics. The answers to the interview questions were compared to the outcomes of the post-photovoice debrief.

Discussions arising from the pre-photovoice interview questions covered a broad range of topics directly or indirectly related to animals. Key themes and topics that emerged from the post-photovoice debrief were more extensive and detailed compared to the pre-photovoice interview. Topics discussed were: Nutrition, husbandry, availability of water, vaccination, diseases and antiparasitic treatment. The role of cattle in cultural and social aspects of the Maasai society as well as human

health themes were also extensively discussed.

Results from this project were brought back to the communities through workshop style community presentations. In response to the concerns about East Coast Fever, bloat and gastrointestinal parasitism identified during the photovoice project, we developed and delivered workshops on the cause, signs, economics, and treatment of these diseases. Additionally in collaboration with animal health workers and local veterinary staff visual information material was developed and distributed to facilitate these workshops. More than 15 workshops were delivered in different regions of the NCA between 2013–2015.

Conclusions: Photovoice provided the researchers with a community generated list of concerns and priorities to improve livestock health and production. It resulted in an expanded breadth and depth of discussions and knowledge transfer, and participants indicated that they found it an empowering process. In addition, the outcomes directly informed us about research and outreach needs and critically re-enforced the importance of a One Health approach. The use of photography provided a new medium for Maasai to express their needs and a focus for researcher–participant communications, thereby facilitating new insights across language and cultural barriers.

Education

P01-001-002

Use of virtual and augmented reality in veterinary education and extension

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Objectives: Virtual reality is defined as an artificial world created using computer generated sights and sounds that allow a user to interact with it. Augmented reality is defined as an enhanced version of reality where the user can overlay digital imagery on the real world using something being viewed through a device, such as a smart phone camera. These techniques have significant potential to enhance veterinary education and extension as both image collection and viewing becomes simpler and cheaper. This presentation covers development of virtual and augmented reality resources for veterinary education.

Materials and Methods: Virtual reality described in this presentation encompasses two separate methods of image collection. The first of these has been described in developing the 4D virtual farm in Australasia. This involves the use of a high quality digital SLR camera with fisheye lens to take six images every 60 degrees on a tripod and a further image taken upwards. These images are then be stitched together to produce a 360 degree image using KR pano. This image can then be placed on a farm map and multiple images taken through time to give the fourth dimension of time. Images were collected in West Australia, Queensland, New South Wales, Victoria and New Zealand with assistance from local veterinary school staff. In addition, 360 video footage is collected using a combination of 6 GoPro cameras connected to a 360 heros 360H6 holder. This is then processed using Videostitch software to provide 360 video content. Augmented reality was produced using the Aurasma application, selecting a relevant trigger image and a relevant video or link applied to this image.

Results: This project commenced as a way to bring the farm back to the “classroom” or space where a student wished to preview and review relevant material. Equally it has been developed to bring the classroom

to a property to aid self-directed learning. It is possible to view the virtual reality experience in equipment such as Oculus Rift or Google Cardboard and it is platform independent so works on any Apple or PC system, including mobile systems. Veterinary students across Australasia are starting to use the 4D farms module in 2016 as a purpose built glossary is completed to work with the 4D farms module. The collaboration of five Australasian veterinary schools allowed the capture of images from a broad latitude and longitude in tropical and temperate environments to illustrate a variety of animal health and production issues. Utilisation of individual 360 degree photographs combined with 360 degree timelapse and 360 degree video allows for a wide range of flexibility in presenting relevant material to students.

Conclusions: Where virtual and augmented reality are designed to aid understanding of specific learning objectives they have great potential to help learners understand what happens in distant environments. This includes bringing the classroom to the farm or the farm to the classroom. As equipment for collecting these images becomes easier to use and cheaper along with cheaper equipment to view these images it is likely that both virtual and augmented reality will become far more utilised in both teaching and extension work in veterinary science.

Education

P01-001-003

Perceived barriers and motivators of UK clinicians and academics towards a national examination in farm animal medicine and surgery

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Objectives: Although veterinary schools in the UK are subject to RCVS accreditation, there is currently no unified assessment between veterinary schools in the UK. With more veterinary schools emerging, it is timely to investigate the feasibility of a 'national exam'. The aim of this study was to gather opinions from relevant stakeholders in UK veterinary schools on the feasibility of developing and conducting a national exam in the area for farm animal medicine and surgery.

Materials and Methods: The study was carried out by interviewing one farm academic clinician involved in final year assessment ('clinician') and one other academic who was involved with the final year examination regulations and processes within each veterinary school ('academic'). Semi-structured interviews were carried out allowing the interviewees to expand on their answers and the interviewer to be flexible in the interview process. The interviews were recorded, transcribed and data analysis was performed using thematic analysis (NVivo 10).

Results: Telephone interviews were conducted with 16 participants lasting an average of 30 minutes, with a range from 17 to 44 minutes. Interviewees reported a large variation in the way the eight veterinary schools examined students in the final year of the course. Interviewees commented that getting all of the stakeholders to agree on a common target outcome would be challenging, not insurmountable, but certainly a significant challenge.

Preliminary analysis demonstrated a marked difference between opinions of clinicians and academics. Clinicians appeared more idealistic and in favour of the suggested national exam compared to academics who primarily considered the logistics and execution and shared concerns with regards to this process.

Conclusions: High quality final year assessments are important to veterinary students, universities and the veterinary profession employing new graduates. Assessments take up significant time and a concerted effort between veterinary schools could improve efficiency. This presentation aims to present the barriers and motivators from relevant stakeholders towards a national exam and provide considerations with regards to the feasibility of such an examination.

Education

P01-001-004

The application of a dairy herd simulation model to teach Dairy Herd Health Management

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Objectives: Teaching dairy herd health management to animal science or veterinary students is complex because it requires the integration of many areas of knowledge (nutrition, genetics, management, diseases, heifer growth, economics,...). In most cases, students have already been exposed to these areas in previous classes. The challenge is to develop education strategies that allow students to learn the integration of all these areas, and to develop comprehensive decision-making abilities. The objective is to present the use of a dairy farm simulation model used to teach a Dairy Herd Health Management class to last year Veterinary students.

Materials and Methods: A stochastic model that simulates physiological events of heifers, lactating and dry cows (biological model for each group) was developed. For each animal, sub models are developed for feeding, diseases, environmental factors, facilities, management, reproductive management, and economics. All these factors follow established equations that account for the complex interactions among them. Default values for variables are provided, but the student can change them. The conditions established are simulated on an animal-by-animal and day-by-day basis. Students work in groups of 3 and generate a farm (typically a 300 milking cow) under specific conditions. Students are provided with a set of different questions (Is fixed-time insemination programs appropriate? Is a short dry period profitable? Is feeding in two groups profitable? What is the effect of reducing by half the incidence of a specific disease?,...). The outcome provides a list of technical and economic indicators essential for the decision-making process, including demographics, production, reproduction, heifers, diseases and economics. Student use the results to answer the question and have to develop a comprehensive strategy (specific recommendations) to improve the profitability of the farm.

Results: While the case-approach is an excellent tool for integrating concepts and developing decision-making abilities, it is often difficult to generate sufficient cases for large classrooms and for different years. The use of computer-generated virtual farms is an alternative tool to expose students to an endless set of scenarios where they can analyze the condition of a farm, propose alternatives, and simulate the technical and economical consequences of their actions. The process is based on self-learning activities and requires the consultation of scientific references to take proper decisions. The analysis of results helps them to relate the technical with the economical outcome. At the end of the semester, students present a set of priorities that need to be implemented in their farm to the rest of the class and a debate is generated.

The implementation of this strategy has eliminated traditional classes

that have been substituted by debates. After a survey at the end of the semester, student satisfaction was very high (overall evaluation of 4.9/5.0 in a 32 student class). Students appreciate the suitability of the method to integrate concepts, and the feeling of "ownership and professional responsibility" with their own farm was highly motivating. The understanding of the economic implications of the different technical decisions was greatly appreciated. The program is currently available at www.dairyfarm.es/simulador. For the generation of a specific class, contact sergio.calsamiglia@uab.es.

Conclusions: The use of the web-based computer model offers a powerful tool to teach Dairy Herd Health Management. The model helps students to learn the integration of the different areas of a dairy farm, and to develop comprehensive decision-making abilities. Students take responsibility in the decision-making process of a dairy farm and evaluate the consequences of the decision taken. The implementation of this educational strategy has been very well received by students.

Education

P01-001-005

Innovative approaches to teaching farm animal clinical skills to veterinary undergraduates

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Objectives: The Bristol School of Veterinary Sciences has recently undergone a major curriculum review. The initiative has included identifying ways to improve the teaching of clinical skills. The work has involved:

- Development of innovative models
- Design of comprehensive supporting learning resources
- Integration of clinical skills teaching throughout the curriculum

Part of the project focussed on the skills used by farm animal veterinarians and the practical and clinical competences expected of graduates entering mixed or farm animal practice.

Materials and Methods: A series of steps were undertaken including identifying a list of farm animal clinical skills, developing teaching and learning resources, and introducing the new initiatives into the curriculum.

The range of practical and clinical skills required of farm animal veterinarians was reviewed through staff and student discussions, reference to the RCVS Day One Competences and equivalent lists, and posting in discussion threads in an online forum (the 'Veterinary Clinical Skills & Simulation' group in NOVICE www.noviceproject.eu).

Opportunities were identified within the curriculum to complement existing farm animal teaching using models in the newly established clinical skills laboratory (CSL). Factors to consider included the challenges learning the skill for the first time on a live animal e.g. internal examinations such as rectal palpation are a 'black box' for teaching and learning (tutors can't watch or give feedback on performance) and whether use of models would help promote student learning. Additionally if a model was to be adopted in teaching, it needed to be realistic enough but value for money, affordable and preferably reusable.

Some models were available commercially while others were developed in-house using materials from a variety of sources including Smooth-Silicon (used for making masks for the movie industry) which proved

readily adaptable to simulate different types of tissue e.g. to practise suturing. Supporting learning resources included a detailed instruction (skill) booklet for each model.

Results: The CSL contains models that help students learn farm animal practical and clinical skills ranging from applying a halter, to taking blood samples, to performing surgical procedures and enhance students' preparation for work-placements on farms, in veterinary practices and in clinical rotations.

Examples of models developed by the CSL team include for ovine skills - 'Mabel' the life-size sheep (to practice turning her over) and 'Alex' a fully articulating lamb (correct malpresentations prior to delivery); bovine skills - including suturing skin, muscle and uterus (each layer fixed to a frame at cow height) to represent a caesarean or displaced abomasum flank laparotomy, intramuscular and intravenous injection and venepuncture; and porcine - piglet handling, application of a snare and jugular venepuncture. There is also a set of 'car boot to cow side' scenarios which allow students to practise selecting equipment and drugs to carry to the patient (without taking the 'kitchen sink' or forgetting something). A booklet at every station describes (in photos and written text) each step to perform a skill, the equipment required, how to reset the station (in readiness for the next student) and some tips from recent graduates (I wish I'd known...). Another approach has involved using commercial bovine rectal palpation models (physical and virtual) as part of a half day practical when students rotate from CSL to PM room (uterine tracts) to the farm to examine live cows per rectum. An isolation station and scenarios enable students to consider biohazards and disease control measures.

Conclusions: An innovative yet pragmatic approach to developing models and associated supporting learning resources has allowed us to incorporate a 'vertical theme' of farm animal clinical skills throughout the new curriculum. The models are used in many taught classes and students are encouraged to return to the CSL for further self-directed learning as and when required, complementing clinical training while promoting and protecting animal welfare. The clinical skills initiative is the work of a dedicated team who have included academic and technical staff, recent graduates and students.

Education

P01-001-006

Digital communication and knowledge transfer for veterinary practitioners and their client animal owners in Ireland

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Objectives: Digital devices and channels are becoming more critical to effective knowledge transfer. To address a dearth of publications on the evolution and uptake of digital technologies, regarding Registered Veterinary Practitioners (RVPs) in Ireland and on their bovine client animal owners (CAOs), the aims of this research are to:

- Compare adoption of digital technology devices among RVPs and cattle owners to general population survey data
- Evaluate digital communication and education by the RVPs and their CAOs
- Determine relative importance of their information sources
- Evaluate patterns of their online behaviour regarding animal healthcare

Materials and Methods: A paper-based survey on uptake of digital devices, communication and online behaviour concerning sources of information and education was conducted by MSD Animal Health (MSD AH) Ireland, of RVPs, by convenience sampling at veterinary meetings in Ireland (≥ 2 per province) between November 2014 and March 2016. Cattle farmers were also targeted with a paper-based survey, comprising the same topics but tailored to farmers, at meetings conducted by MSD AH and other organisations (Teagasc), between July 2015 and March 2016. RVP uptake of websites and social media was evaluated. Digital adoption, communication and online behaviour data were gathered from the surveys of the target personas and the general population for comparison. Survey results were statistically analysed using a software package (STATA®) and tabulated.

Results: Website ownership or social media activity by RVPs varied between 50-80%, significantly associated with practice size. Facebook usage was correlated to presence of a website. Websites are used to market the practice through organic search, but also to educate their owners on animal care, more commonly for companion animals than ruminants. RVPs most commonly communicated information to clients via SMS text followed by hard copy and email. Only one third of vets communicated with their clients via social media. The majority of RVPs own a laptop, with approximately 2/3 owning a smartphone and only 1/5 owning a tablet device. Whilst almost 1/2 of vets use search engines weekly, colleagues, journals and conferences ranked in descending order as information sources.

The majority of cattle owners viewed the RVP as the most importance source of animal health (AH) information with the Irish Farmers Journal (IFJ) considered the next most important. The IFJ was the most frequently used website for seeking AH information after Google. Farmers significantly preferred email over text, phone or mail as the communication method from their vet for herd health information, though few read e-newsletters. Approximately 3/4 of farmers owned a smartphone, 1/2 a laptop and only 1/4 a tablet device. Smartphones were more or less equally used for email, telephony, text and internet surfing but less for social media. 1/3 of farmers read their emails every day and an additional 1/3 a few times per week. Social media usage by farmers was significantly lower than the general population.

Conclusions: The RVP has a significant role to play as a trusted educator on herd health. To capitalise on this position, they must consider the most appropriate means of dissemination of credible material across the preferred device and channel. Email may replace text as the preferred communication tool for farmers as the smartphone dominates. For successful information transfer, media must encourage a higher engagement rate with content than currently exists with e-newsletters.

Education

P01-001-007

Using a Massive Open Online Course (MOOC) to increase the reach of veterinary education

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Objectives: Transferring new and existing knowledge and technological advances across the globe will be critical in feeding 9 billion people by 2050. The US has a long-established, highly successful model with the Agricultural Extension System to disseminate information to producers

and veterinarians. We explored how this model could be expanded to have a global reach by applying novel, online learning technologies, through Massive Open Online Courses (MOOC).

Materials and Methods: We developed a six module MOOC for the Coursera® platform entitled Sustainable Food Production Through Livestock Health Management that was delivered in the Spring of 2015. Each of the six modules explored a single aspect of how disease impacts the sustainability of food production. As part of each module, participants participated in discussion forums around their areas of interest and completed knowledge assessments. A capstone project was available for participants that wanted to deepen their learning. The course was provided at no cost to participants.

Results: A total of 8847 people joined with 5238 people completing at least part of the course. Participants were from 165 different countries with 35% from emerging economies. Between 379 and 1049 people completed the quiz each week with a reduction in number each week over time. The number and diversity of people reached by this program were much greater than though traditional educational platforms. Participation and completion rates are above the median for other courses at our institution.

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Conclusions: The data suggest that many people are interested in learning but choose to select the material that they want and may not be interested in traditional "course" based learning. This fits with a macro trend of a preference for competency development programs instead of credential granting training. The MOOC format while not perfected, creates an intriguing way to disseminate knowledge outside of traditional channels to increase the impact of institutions.

Education

P01-001-008

Utility of an online learning module for teaching disbudding in dairy calves, including cornual nerve block application.

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Objectives: The objective of this study was to determine if an online, interactive module could teach naïve participants appropriate disbudding technique, including application of a cornual nerve block, as compared to small group, hands-on learning.

Materials and Methods: Thirty-four student volunteer participants, with no prior experience with application of a cornual nerve block, were randomly assigned into two training categories; online, or hands-on. Volunteers were students enrolled in year 1 or 2 of the Doctor of Veterinary Medicine program (DVM) at the Ontario Veterinary College

(OVC), graduate students, or undergraduate students at the University of Guelph.

Hands-on learners were taught in groups of 2 to 4 by a registered veterinary technician (RVT), responsible for similar teaching in year 4 of the OVC DVM curriculum. An established protocol was followed, where students watched a demonstration disbudding of one calf, followed by the opportunity to practice on a single horn bud, with as much assistance as needed. Online learners were given access to an interactive training module that was developed based on the established hands-on protocol.

Evaluations were done in groups of 3 to 4 students, with at least 1 student from each treatment group. All evaluations were by a single, blinded, evaluator. Participants were scored on background knowledge by a written quiz, and scored on handling, block technique, and disbudding technique, by an established rubric. Time taken for each step of disbudding was measured, and participants were asked to rate their confidence on a score of 1 to 5 both before and after evaluation.

Calves were considered to be inadequately blocked if they performed any of seven established behaviors during the first five seconds of disbudding iron application, and a 'rescue' was performed by the RVT, who re-blocked the calf.

Results: Twenty-five percent of calves in the online group were considered inadequately blocked, and required a 'rescue'. No calves in the hands-on group were considered inadequately blocked. This result was not statistically significant (Fisher's Exact, $p=0.126$).

Online participants had significantly higher written test scores ($p=0.038$), but significantly lower handling scores ($p=0.023$) and disbudding scores (0.041). Technical scores for block application were not significantly different between groups.

Time taken was significantly longer in the online group; an additional 16.50 sec for blocking ($p=0.025$) and an additional 33.32 sec for disbudding ($p=0.010$).

Online participants were significantly less confident prior to evaluation (-0.70 , $p=0.001$), however, post-evaluation confidence scores were not different between groups. Online participants tended to have a greater change in score between pre- and post- evaluation ($+0.49$, $p=0.063$).

Conclusions: Online training was moderately effective at teaching naïve participants to successfully block and disbud a dairy calf. Although no statistical difference in block efficacy was seen, the numerical tendency for online learners to be less effective might approach significance if the trial involved more participants.

Online learners were less confident, had poorer handling skills, and took more time. After the evaluation, online learners' confidence score tended to increase compared to the hands-on group's, suggesting the importance of actual experience. Best practices for may involve a combination of both online and hands-on learning.

Education

P01-001-009

Outcomes from a training program to attract, upskill and retain veterinarians in the Australian dairy industry

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Objectives: Rural veterinary practices have found it difficult to attract and retain early to mid-career veterinarians risking a reduction in rural veterinary services, particularly in dairying areas where veterinary expertise is critical to farm productivity. The first two rotations of a three-year rural veterinary training program led by the University of Melbourne has provided the Australian dairy industry with a much needed boost in the availability of skilled dairy veterinarians who are now embarking on rewarding and productive career paths in the agricultural sector.

Materials and Methods: The training scheme sourced young veterinarians with an interest in dairy medicine and production to undertake post graduate training whilst embedded in one of six large, mixed veterinary practices located in the major dairying regions of Victoria and Tasmania. They were enrolled in a Master of Veterinary Studies (by course work) and a Master of Veterinary Science (by research) over three years. During that time they conducted farm-based research useful to the dairy industry; increased their capability to contribute to industry by skills acquisition through advanced clinical training; and acted as tutors, role models and mentors to veterinary students. The advanced training led to an understanding of the integration of farming systems within and beyond practice to enable them to develop expertise in whole-farm production programs such as fertility, herd health, mastitis & animal welfare. During training, the students were exposed to a wide selection of scientific and dairy industry experts, significantly broadening their industry knowledge and skills.

Furthermore, all participants were required to present their research results and other project findings in a variety of forums, including local and regional farmer meetings, the Australian agricultural media and various national and international scientific meetings and journals. This ensured the wide dissemination of practical outcomes from their farm-based research which in turn has encouraged students and veterinarians to consider career opportunities offered through the dairy industry in a variety of Australian regions.

Results: A major outcome of the program has been the number of important local animal health and production problems studied by the postgraduate students as part of their training. Research was conducted with full academic rigour and support and has led to economically viable solutions to many of the issues. The projects have included identifying management factors that could reduce prevalence of anoestrus in Victorian dairy herds; quantifying reasons for dairy herd bull infertility; identifying effective and practical colostrum management guidelines and factors affecting colostrum quality; confirming the presence of anthelmintic resistant parasites in Victorian dairy cattle; use of daily weight measurements as a predictive tool for reproductive performance; and the prevalence and associated risk factors of heifer mastitis in Victoria.

Each resident has published peer reviewed manuscripts, delivered extension activities and submitted reports detailing their activity over the three years. University of Melbourne veterinary students undertaking a compulsory two week dairy rotation in one of the six partner practices have recorded overwhelming approval for the scheme, highlighting the benefit of seeing dairy practice with enthusiastic and motivated young dairy veterinarians.

In practical terms the program has trained seven dairy residents with six awarded MVS/MVSc degrees and one converted to a PhD. Another graduate commenced a PhD at the completion of her Masters study and the remaining graduates have accepted consulting roles with dairy practices or the wider agricultural industry.

Conclusions: The program has provided a unique opportunity for professionals to develop skills and knowledge across the complete production chain from farm gate through the manufacturing process to the consumer, equipping them to make contributions to the industry, both in research and in knowledge transfer.

Most importantly it has allowed the major funding bodies to leverage their

investment for the benefit of the whole industry rather than a specific section or group.

Education

P01-001-010

Reverse engineering in animal science: visual perception of body condition score differences in three-dimensional models of cattle

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Objectives: The evaluation of body condition score (BCS) is widely used in the management of cattle. Changes in BCS reflects mobilization of energy reserves, with direct consequences for production, reproduction, and animal welfare. The rapid generation of 3D models can be an important alternative to conventional strategies for the evaluation of BCS. However, in most cases, the obtained 3D models are not colored, which may condition users perceptions. The aim of this research study was to investigate the combined used of scanning technologies and 3D modeling to access its effects on the visual perception of BCS.

Materials and Methods: Conventional photographs and 3D data of the lumbar, pelvic, and tail head regions were obtained from Holstein-Gir crossbred dairy cows (n=67). Digital color photos were taken from the upper-rear view of each cow, while 3D data were recovered by structured infrared light scanning of the same area, using a sensor equipped with built-in infrared projector and infrared and RGB video cameras. A 3D model of each cow was created using a real-time 3D scanning software (ReconstructMe 2.0), and edited using an open-source software for processing 3D triangular meshes (MeshLab). The 3D models were then rotated in the X-Y-Z axes to provide a view of each cow equivalent to that used to take the photo, and snapshots were made and stored as TIFF files. Three experienced evaluators (E1, E2, and E3) independently assigned BCS to the cows using the photos and the snapshots of the 3D models, with the same reference procedure and scale (1 to 5), without knowing the identification of the cows nor the relationship between pictures (blind evaluation). The main effect of the image type (photo or snapshot) and the effects of cow, evaluator, and their interactions, were included in the statistic model, and analyzed with the GLM procedure of the SAS software. The association between BCS outcomes was calculated using the Pearson's correlation method. The correlations were compared using a package for R programming language (comparingcorrelations.org). Data are described as mean \pm SEM. A p-value of 0.05 indicated statistical significance.

Results: There was no difference in average BCS determined using the 3D model snapshots or the conventional color photos (2.85 \pm 0.21 vs 3.03 \pm 0.22, respectively; P = 0.79). The BCS were affected by cow (P = 0.04) and evaluator (P = 0.04), but there was no interaction of image type and cow (P = 0.46) or evaluator (P = 0.07). The correlations between BCS values for the evaluators were not affected by image type (R = 0.79 vs R = 0.84 for E1 x E2, P = 0.41; R = 0.85 vs R = 0.91 for E1 x E3, P = 0.13; and R = 0.79 vs R = 0.81 for E2 x E3, P = 0.76; for photos or snapshots, respectively).

Conclusions: The use of virtual models constructed by 3D data acquisition and 3D modeling, despite lack color information, does not

interfere in the perception of BCS, and thus can be used for educational/training purposes. These models have many potential advantages, including preserving the correct proportions of the original models, and the possibility of being rotated in the virtual space and explored in different angles. Moreover, they can be used in enhanced reality, or 3D printed to provide tactile experience, e.g., to mimic the BCS evaluation techniques that associate visual and tactile information.

Acknowledgements: Embrapa and Fapemig.

Education

P01-001-011

The Group of Study, Research and Extension in Goats and Sheep (GEPECO) of the Universidade Federal Fluminense, Brazil

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Objectives: The group aims to support the consolidation and profitability of the sheep and goat production at Rio de Janeiro State. To achieve this goal, we have been working with the development of special skills in students of veterinary medicine with the association of research and extension activities in the segment.

Materials and Methods: The Group of Study, Research and Extension in Goats and Sheep (GEPECO) is formed by undergraduate and postgraduate vet students, coordinated by associate professors of the Veterinary Faculty. The students develop skills in the sheep and goat unit of the Farm School and later they exercise along the extension actions in farms assisted by the group. At each meeting, a new practice theme is presented to students. The main themes are: (1) facilities, ambience and Animal Welfare; (2) Strategic Parasite Control and vaccination protocols; (3) Nutrition and pasture management; (4) Reproductive management; (5) Medical Clinic and routine surgical procedures; (6) Breeding and profitability of the activity; (7) Industrial processing of meat and milk - visits to slaughterhouses and dairy factories. Prior to visiting the property, the group discusses about the procedures to be performed. All visits are guided by a postgraduate vet student or professor who deals with the farmer and performs the activities with the help of the students involved. Furthermore, the students also learn the language used with it and the different measures may be adopted in accordance with the socio-economic realities encountered. Thus, the extension actions encourage the adaptability of the students, future professionals, teaching him how to act in front of different circumstances, stimulating creativity and confidence of them. It also has been done applied research, conducted by postgraduate students, with farm partners aiming to solve issues reported previously by them and minimizing production costs.

Results: The GEPECO has trained 43 vet students since 2008. It has also participated and has exhibited scientific findings on conferences. Moreover, it has helped to make up for the lack of existing technology in the sheep and goat production through of the extension actions. Nowadays, the group assists eight farms, from larger commercial to familiar production systems with important social role. Most of them are located on the outskirts of the Farm School. The most challenges for farmers are related to labor hand on the property and nutrition issues. Among the activities developed by the group in these farms, the examination of eggs per gram of feces in association with Famacha[®] technique for

the deworming control strategy have been done. Vaccination procedures and changes in some management were also successful to control outbreaks of infectious diseases. The use of hormonal protocols in the breeding and out-breeding season in association with the ultrasound diagnosis of early pregnancy has helped to optimize the reproductive gains. Furthermore, monitoring of the animal production and culling procedures have also been contributed to improve genetic gains in each farm. At long last, students will incorporate these techniques to use in their professional life, as well as the farmers are benefited by optimizing the production system.

Conclusions: The GEPECO collaborates in the development of skills and professional insertion of students of veterinary medicine, in food production, in the rural revenue of sheep and goat farming and keeping people in the countryside.

Comments: The authors believe that the inclusion of this abstract about the GEPECO, in the education topic, will be important to generate discussion and take criticism and suggestions on the same.

Education

P01-001-012

Teaching on farm biosecurity and welfare to future veterinarians, a professional practical training at UNIBO

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Objectives: In an effort to prepare the next generation of veterinarians with the wealth of knowledge they need, the Department of Veterinary Medical Sciences (University of Bologna), in collaboration with the AUSL Public Health Area, included on-farm biosecurity training in the fifth year of the veterinary curriculum to prepare the students to think holistically about different livestock

Materials and Methods: During the activity, each student visit two properties: one small ruminant and one cattle farm and use the biosecurity and welfare checklists that are commonly employed during farm's official controls. Students are encouraged to ask questions to the owner and/or to the manager and collect samples for laboratory analyses which are subsequently analysed during laboratory activities. At the end of the activity, they turn into a communications exercise preparing and presenting a biosecurity plan that is given to the farmer.

Results: Sixty one farms been visited in 2014-2015 mainly in Emilia Romagna region. Small ruminant properties were mostly small sized dairy farms within 25 to 50 animals, but hobby and beef farms were also included. Cattle properties were mainly single or multi sides dairy farms with 200 to 1000 animals. Different issues related to livestock movements, management of equipment, waste, feral animals and pest and feed and water have been identified for each visited property. One of the main issues related to small ruminants was that 60% of the farms can not count on specialised veterinary assistance due to a lack of professionals working on this field in Emilia Romagna region. Environmental factors such as NH3 concentration is not perceived as a risk factor for respiratory diseases leading to a wide use of vaccines and

antibiotics to prevent and treat such disorders. Farmers are frequently not aware about SRLV contribute to new born mortality, mastitis, respiratory and CNS disorders even if more than 60% of small ruminant properties resulted infected.

Conclusions: The UNIBO practical training on biosecurity and welfare resulted in an increased awareness of last year students on the management measures that can be put in place to reduce the use of therapeutics and vaccines with positive impacts on farm economy and food safety. At the same time it consolidated the collaborations of the Academia with veterinary practitioners, farmers and veterinary officials in defining educational priorities for future veterinarians.

Education

P01-001-013

Virtual visits to production units and real time information systems as a Zootechnics learning tool in the undergraduate studies of the FMVZ

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Objectives: The aim of this project is to promote, increase and generalize the quality of teaching and learning of the future professionals of Veterinary Medicine and Zootechnics, through new approaches, logics and technologic and computer resources that will not only increase the responsibility of students in their learning process, but also contribute to a shared commitment between academics and students.

Materials and Methods: IP cameras in the area of the milking parlours for cows and goats at the CEIEPAA of the FMVZ-UNAM were installed during the development of the project. The cameras were interconnected with a real time transmission system, which in turn is available in the website of the Medicine, Zootechnics and Academic Extension Secretariat, as well as with a video archive system, which can be consulted in the same website. With a previous registration at the internet page, both the student and the professor can look at the real time transmission of the captured procedures. On the other hand, short-clips edited videos are also shown

Results: The results are in the internet page at the servers of the FMVZ-UNAM, following the link to "Departamento de Zootecnia", with the following website: <http://www.fmvz.unam.mx/zootecnia/>

In this internet page, one should follow the instructions depending on the computer's operating system:

When the complement is installed, the following screen will show, asking for:

Username ("Nombre de usuario"): basico (lower case, no accents)

Password ("Contraseña"): zootecnia (lower case)

Then, click the "LOGIN" button.

The user's interface is the following:

By default, only one camera is selected (as shown in A figure). The visualization menu is shown at the bottom part, allowing 4, 5 or more cameras to be selected at the same time (as shown in B figure).

Cameras 1, 2, 3, 4 and 9 are located in the milking parlours of cows, whereas cameras 5, 6, 7, 8 and 10 are in the milking parlours of goats.

The schedule for milking at the CEIEPAA is the following:

Cows: 8:00 am and 4:00 pm

Goats: 8:00 am

From the same website, there is an automatic access to the websites of FMVZ, the Medicine, Zootechnics and Academic Extension Secretariat and to the webpage of each of the seven Learning, Research and Extension Centre of the University.

Conclusions: The teaching of Veterinary Medicine and Zootechnics could be improved using information technologies and having access to these type of computer tools. Thereby, the students with the supervision of professors can reach to certain conclusions which are based in real systems of production, enabling them to develop a critical and analytical sense to make recommendations and increase the productivity in their future field of work. 1, 2 y 4

Comments: This work is part of the PAPIME PE201513. UNAM. Project "Virtual visits to the production units and real time information systems, as a Zootechnics learning tool in the undergraduate studies of the FMVZ"

Education

P01-001-014

The gamification of the classroom, or how to learn dairy management through playing

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Objectives: In the Bologna educational systems the student is in the center of the learning process, he has to work more independently, and an experienced-base environment is highly recommended. Case studies are excellent to develop thinking abilities and integrate concepts, but it imposes a burden on the instructor to generate a large number of cases and to grade them. The objective of this paper is to present a web-based dairy farm simulation model designed to be used in introductory courses of Dairy Herd Management in Veterinary and Animal Science studies.

Materials and Methods: The model is programmed in Java and is accessible by internet. Modeled events are based on physiological functions of cows and work at random around predefined averages and ranges. The program has three levels of interaction: the administrator that coordinates the system; the instructor that controls all conditions within his class and can visualize each individual student activity; and the student that controls all actions in his farms. At the beginning of the course, the instructor defines all parameters of the farms that will be assigned to students (farm size, production level, dietary ingredients available, fertility, detection rate, incidence of diseases,...) and the program generates a unique farm for each student within these limits. Actions taken by students include: heat detection, insemination, dry off, diet specifications, formation of groups and transfer of animals between groups, colostrums and milk feeding, weaning, treatment of diseases, milk withdrawal if antibiotics are used, buying and culling of cows or heifers, among others. The daily output provides information on milk production, economic performance and counters of errors incurred as feedback for students. The final grade is calculated automatically. Additional exercises based on their own farm (i.e., calculate required space for cows, land for forages production or manure spreading,...) can also be implemented.

Results: The web-based computer simulation model is an excellent strategy to generate an unlimited number of farms to allow students to take decisions individually and assume their consequences. The program can be used by the instructor as it is in introductory classes, but it may also be used in parallel to formal classes to illustrate specific concepts (i.e., the instructor can cause a change in forage quality or modify fertility and make students identify the problem and discuss the consequences at the dairy farm level). The program has been used for the last 5 years by undergraduate and Veterinary School students in different universities with excellent evaluations of the learning process from student surveys. In a non-planned exam conducted 2 weeks prior to the final exam, students were 5 times more likely to respond correctly to the same type of questions about dairy cows than other species taught in the same semester. The program is written in Catalan, Spanish and English. For information, connect to www.dairyfarm.es. Information provided includes a Manual for Instructors, a Manual for Students, a Quick-start guide, and two 12-minute videos with instructions for student and one 10-minute video with instructions for the instructor on how to use the program. The program is free of charge. To activate an account as instructor, send a request to sergio.calsamiglia@uab.es. The estimated amount of work for an introductory class is two-hour lecture and about 6 to 8 h of individual work per student.

Conclusions: The web-page dairy farm simulation program is an excellent tool for teaching introductory courses in Dairy Herd Management where students become in the center of the learning process by being responsible for all activities that take place in a dairy farm. It allows instructors to generate as many farms as students, and the grading is automatic.

Education

P01-001-015

Homepage about the fertility of the healthy cow with two animations: the cycle of the cows heat and the development of the gestation

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Objectives: In 2010, the Swissgenetics company developed the idea to provide a homepage about the fertility of the healthy cow for their customers – the farmers and also for students of the veterinary medicine. This as a possibility to increase the customer loyalty. The homepage had to be presented in German and French, later maybe in English, too. The number of visitors should reach at least 5% of the total number of accesses on www.swissgenetics.ch

Materials and Methods: A mind map was generated for each chapter after discussion with veterinarians and agronomists. With aid of the common literature, the themes were deepened. All texts were written by a veterinarian specialised for cows. For each theme, pictures, films, interviews or animations (the cycle of the cows heat and the development of the gestation) were generated or added. The program used for the construction of the website was TYPO3.

The website has been promoted in lessons at agricultural schools, newspaper advertisement and newsletters.

Results: www.die-fruchtbare-kuh.ch (in German) and www.la-vache-fertile.ch (in French)

The cycle of the cows heat and the development of the gestation are shown in two extraordinary animations. Seven chapters were elaborated: the fertile cow, the cow with disturbances of fertility, heat detection, insemination, birth and breeding, herd management and signals of cows. On the front page, there are rotating themes of articles, signals of cows, news and the two animations.

With a photograph contest about signals of cows in January-March 2015, the number of visitors increased intensely. Since then, twice per month, a signal of cows is analysed in the newsletters of Swissgenetics and of the homepage. A professional film about the birth of the calf brought more than 300'000 visitors (channel of youtube).

Conclusions: To establish such a homepage, several years are needed. With special designs like the cycle of the cows heat and the development of the gestation, the homepage becomes inimitable. Regular promotion is necessary. With the contest for the customers on a popular subject, the number of visitors increased rapidly. It stays at the aimed level thanks to the rotating information on the front page and its professionalism.

Comments: Swissgenetics, Switzerland

Education

P01-001-016

Cattle diseases: physical sign profile similarities using sign frequency pattern recognition

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Objectives: An important step in the formation of a list of differential diagnosis in clinical practice is the identification of diseases that are similar in clinical profile to the leading hypothesis. The objective of this study was to generate a similarity chart and an ordered list based upon sign profiles and frequencies within cattle diseases.

Materials and Methods: The data contained within the diagnostic expert system Bovid 3 was used as the source data. This data was derived from expert opinion and contained the sign frequencies for over 1100 cattle diseases across a comprehensive range of clinical signs. Only physical signs were used in the analysis. The data was entered into a software program which used a simple linear nearest neighbour comparison based upon the differences between the disease sign frequencies to compute the similarities. The outcome is presented in a dendrogram format and a list.

Results: The output was a chart indicating the similarity of the diseases in a hierarchy and an ordered list with adjacent diseases being the most similar to each other. Each disease is placed next to the disease with the greatest similarity according to the array of sign frequencies within the disease profile. The order of the diseases indicates the relative profile differences between the diseases. The relative prevalences can be represent either numerically or by colour coding. Alternatively the database of diseases can be limited to common diseases only.

Conclusions: This study has provided a resource for differential diagnosis in clinical practice and a classification of diseases which could be used in problem based learning. The dendrogram represent a taxonomy of cattle diseases according to the array of physical sign frequencies within the disease profile.

Emerging Diseases

P02-002-070

Dynamics of Anti-Schmallenberg Virus Antibodies in a Naturally Infected Dairy Cattle Herd

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Objectives: In late 2011, the novel insect-transmitted orthobunyavirus Schmallenberg virus (SBV) emerged in Central Europe. Aim of this study was to investigate the development of SBV seroprevalence and duration of immunity in naturally infected animals in one Herd in the years 2011 - 2014.

Materials and Methods: All cows older than 24 month were blood-sampled in December 2011 or January 2012 (total of 311 animals), again in January 2013 (331 animals), in December 2013 (324 animals), and in December 2014 (330 animals). From 122 cattle blood samples were available from all four sampling dates. The cows included in the study were kept indoors year-round, but all animals younger than 24 month are kept outside. All samples were taken by jugular venipuncture by the responsible farm veterinarian in the context of the health monitoring program of the farm under the control of the Bovine Health Service (Chamber of Agriculture for North Rhine-Westphalia) and analyzed by a commercially available SBV antibody ELISA (ID Screen® Schmallenberg virus Competition, ID vet, France), using the recommended cut-off of 40% relative optical density compared to the negative control (S/N, %). All samples taken from an animal were tested on the identical ELISA plate.

Results: In December 2011, in 74% of the tested animals SBV-specific antibodies were detectable. Additional scattered seroconversions were observed between the 2011 and 2012 vector seasons, thereafter all seronegative animals remained negative. Until December 2014, the intra-herd seroprevalence decreased to 58%. This lower number of animals with measurable anti-SBV antibodies is most likely caused by the missing infection of the young stock, especially as the year with the highest virus circulation was 2011 and the proportion of animals born after 2011 among the blood-sampled increased from 2013 to 2014 since older animals are gradually replaced by the offspring. A total of 122 cows infected presumable in autumn 2011 were sampled every year, 9 of them became seronegative until December 2014.

Conclusions: Consequently, though SBV-specific antibodies were detected in about 90% of the monitored animals for more than three years, a lifelong antibody-based immunity is not expected in every animal. The loss of anti-SBV antibodies in individual animals combined with the missing infection of young stock results in a declining herd seroprevalence and increases the risk of a renewed virus circulation to a greater extent within the next years.

Emerging Diseases

P02-002-071

Schmallenberg virus detection the bull semen in Poland

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Objectives: Detection of Schmallenberg virus (SBV) in bull semen raised a concern in genital transmission to susceptible germplasm recipients. SBV RNA was detected yet only in bovine semen, limited studies in experimentally infected ram and buck failed to detect the virus. Recent study showed infection of transgenic mice and cattle after subcutaneous injection with SBV positive semen. Viral RNA has been detected in the bull semen with variable dynamics even after three months from seroconversion. The objective of the study was to evaluate SBV presence genetic material in bull semen in Poland.

Materials and Methods: Semen samples from 131 bulls collected between 2013 and 2015 from four herds spread across the country. The serological status of individual animals was unknown. SBV DNA standard (a product of rt-RT-PCR- DNA strand, complementary to the RNA segment of SBV-S template) was prepared in order to optimize the protocol and evaluate the efficiency and sensitivity of different RNA extraction methods. Four extraction methods (3 commercial silica membrane-based methods and Chomczynski based method of TRI extraction) were assessed for level of β -actin (internal control of amplification) and recovery rate of prepared DNA standard detected in rt-RT-PCR in semen. Tenfold dilutions of DNA standard in TE buffer and SBV DNA-spiked semen were prepared and after extraction (each method) RT-PCR was performed. Two pairs of primers designed to detect SBV-S segment and β -actin gene as internal control were used in the standard real-time RT-PCR developed at FLI (Riems, Germany) using AgPath-ID One-Step RT-PCR Reagents (Ambion, Applied Biosystem) kit in StepOne Real-Time PCR system (Life Technologies). The reaction was considered positive when a Ct value < 40 was obtained both for SBV-S and β -actin.

Results: Seven semen samples out of 131 (5,3%) tested were positive for the presence of SBV RNA with Ct values between 34.5 and 37.2. Positive samples originated from two out of four herds and accounted for 4.8% (3 out of 63 bulls) and 13.3% (4 out of 30 bulls) of the samples tested from those two locations. SBV genetic material was detected in semen collected in 2014 except one from end of 2013.

From the four tested extraction methods, Viral RNA Mini Kit (Qiagen) was selected as the most sensitive method allowing detection of 10 copies of viral DNA per reaction and PCR efficiency of 104% in standard DNA-spiked semen. The extraction method resulted in the lowest levels of Ct values for β -actin in the commercially diluted semen sample which probably due to some inhibitors which affected negatively other extraction methods tested. And finally, Viral RNA Mini Kit needed only 140 μ l of semen what is also significant when testing semen samples frozen in straws. The detection limit of 1 copy per reaction and efficiency 93.1% was established for the DNA standard diluted in TE buffer. This demonstrates that semen extraction method which was chosen and semen as a potentially inhibiting medium did not decrease substantially the detectability of SBV.

Conclusions: The presence of viral RNA in the semen suggests quite recent SBV outbreaks in the two bull herds, however the source has not been identified. This discovery confirms that even in low virus circulation in vector and high seroprevalence new infections occur. The presence of virus genetic material in the tested semen does not implicate directly its infectivity. However, the risk of SBV transmission through artificial insemination or mating should not be ignored and the emergence of new outbreaks in naïve herds should be considered. Since SBV was detected in gonadal tissues virus infection in aspect of germplasm quality should be considered.

Emerging Diseases

P02-002-072

Zulvac® SBV, a vaccine against Schmallenberg virus in breeding sheep that reduces viremia and transplacental infection

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Objectives: The Schmallenberg virus (SBV) was first identified in Germany (2011). SBV is transmitted mainly by biting midges (*Culicoides* genus) and can infect cattle, sheep and goats. SBV rapidly spread in Europe causing mild transient disease in adults (i.e. drop in milk production, fever, diarrhea) but main losses are associated with the infection during pregnancy due to SBV ability to cross placental barrier causing congenital infection.

The aim of this study is to evaluate the efficacy of Zulvac® SBV in breeding sheep by evaluating the reduction of viremia and transplacental infection associated with SBV infection during the first half of pregnancy.

Materials and Methods: The Zulvac® SBV vaccine is a suspension for injection intended for active immunization of cattle and sheep against SBV. The active substance is the inactivated SBV strain BH80/11-4 adjuvanted with aluminium hydroxide and saponin.

Thirteen (13) ewes at breeding age were vaccinated according to the vaccination schedule: two doses of 1 mL three weeks apart by subcutaneous route in the axillar region. Fifteen (15) ewes were left unvaccinated as controls.

Fourteen days after the second dose, animals were naturally mated and approximately at 50 days of gestation the animals were challenged with SBV inoculum. After the experimental infection, blood samples were collected to evaluate the presence of genome in the ewes' blood and amniotic fluid samples were collected to detect the presence of virus genome. Ewes were also monitored for the appearance of clinical signs and hyperthermia.

Twenty-five days after challenge (approximately 75 days of pregnancy) ewes were euthanized and samples were taken from ewes and fetuses to assess the absence of viremia and prevention of transplacental infection.

Results: Before challenge, animals vaccinated with Zulvac® SBV vaccine presented neutralizing antibodies against SBV whereas the unvaccinated remained negative.

After challenge, neither the vaccinated nor the control ewes demonstrated any clinical signs associated with SBV infection, with the exception that control ewes presented a higher rectal temperature 4 and 5 days post-infection compared to the vaccinated animals.

There was a prevention of viremia in 92% of the vaccinated ewes (SBV genome was only detected in 1 vaccinated animal for 1 day), whereas 93% of the unvaccinated ewes developed viremia.

SBV genome was not detected neither in the amniotic fluid nor in any of the fetuses' samples from vaccinated ewes, while SBV genome was detected in the amniotic fluid and/or fetuses' samples of 45% of the unvaccinated ewes.

Conclusions: Zulvac® SBV administered in sheep at breeding age has been shown to prevent viraemia associated with SBV infection in 92% of

the vaccinated animals and prevent transplacental infection in 100% of the vaccinated and infected ewes.

It can be concluded that Zulvac® SBV vaccine provides a prevention strategy against SBV as this is the first vaccine against SBV in cattle and sheep centrally authorized in the EU.

Emerging Diseases

P02-002-073

Genotyping of Leptospira Strains Isolated from Cattle in Argentina

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Objectives: Leptospirosis is a worldwide zoonotic infectious disease which induces abortions, stillborns and weak offspring in cattle. Bovine leptospirosis is frequently diagnosed in our laboratory by serology, bacteriology and molecular methods. Because of the high genetic variation present in this spirochete, the molecular typing of the prevalent pathogenic strains is important for the determination of surveillance, prevention and control strategies according to the actual epidemiological scenario. The purpose of this study was to molecularly genotype the pathogenic *Leptospira* strains isolated from clinical cases of cattle in Argentina.

Materials and Methods: *Leptospira* was isolated from abortions produced in dairy farms in Buenos Aires Province. Fetus was collected and processed within 24 hours in the laboratory. In other case, kidney was collected from slaughterhouses. Immunofluorescence was done and was positive in all cases. Genotyping was performed using the Multiple-Locus Variable-number tandem repeats Analysis (MLVA). This technique was used with one set of oligonucleotides specific for the pathogenic strain *Leptospira interrogans sensu stricto*. The following loci were used to discriminate the strains, VNTRS: 4, 7, 9, 10, 19, 23 and 31. Fifteen bovine-isolated strains were genotyped by MLVA.

Results: The strains were genotyped by MLVA obtaining the following:

1- Two genotypes of *Canicola* Portlandvere and eight genotypes of *Pomona* Pomona isolated from bovine abortions; 2- One genotype of *Hardjo* Hardjoprajitno and two genotypes of *Canicola* Hond Utrecht IV, from bovine kidneys from slaughterhouses; 3. Two genotypes of *Icterohaemorrhagiae* RGA isolated from kidneys of calves.

The results of this experiment show the wide range of genotypes present in the isolated *Leptospira* strains of in Argentinean cattle.

Conclusions: In conclusion, it is recommended to use detailed surveys in all endemic regions in order to detect new genotypes and to analyze if new genotypes should be included in the current vaccines to prevent bovine leptospirosis according to the country or region.

Emerging Diseases

P02-002-074

The dynamics of Schmallenberg virus circulation in *Culicoides* spp. midges after the transmission of virus to Poland in 2012.

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Objectives: The Schmallenberg virus (SBV) is a novel arthropod-borne pathogen transmitted by blood sucking female midges of *Culicoides* genus which has emerged in Europe in 2011. In August 2012, SBV was first detected in cattle and *Culicoides* spp. midges in Poland. Subsequently, the circulation of SBV was detected in over 10% midge pools collected during the 2012 activity season. The virus has been recovered also from the nulliparous females which may suggest transovarial transmission. The further studies were performed in order to determine the prevalence of SBV in subsequent years and the possibility of SBV overwintering in the *Culicoides* vector.

Materials and Methods: *Culicoides* spp. midges were collected using Onderstepoort and Model CDC 1212 John W. Hock Company UV light traps set in 23-26 evenly distributed locations in the country between 2013 and 2015. Most traps were located in a proximity of domestic ruminant farms, while two traps were placed near the rest places of European bison (*Bison bonasus*) at the breeding reserves of Białowieża National Park (BNP) at Białowieża Primeval Forest. The midges were separated and pooled (mean 24 individuals) according to species, parity status, location and date of collection. Over 3000 pools were tested. The insects were homogenized in RLT Buffer using Lysing Matrix D Tubes with ceramic beads in Ryzolizer. Total RNA was extracted using RNeasy Mini Kit (Qiagen) in the automatic station Qiacube (Qiagen). Two pairs of primers designed to detect SBV S segment and a fragment of 18S midge gene as internal control were used in the in-house optimized real-time RT-PCR using AgPath-ID One-Step RT-PCR Reagents (Ambion, Applied Biosystem) kit in StepOne Real-Time PCR system (Life Technologies). The SBV positive midge pools were retested in order to verify the result.

Results: The percentages of SBV positive *Culicoides* pools were 0.9% (13/1401) and 1.1% (18/1569) in 2013 and 2014, respectively. The mean Ct values high and reached 39.4 and 34.7 in those two years. SBV was detected in 2.8% (8/283) midge pools collected in 2015 with the mean Ct value of 38.6, however the testing has not been finished by the time the abstract was prepared. SBV RNA has not been detected in any of the midges from BNP in 2014 and 2015.

Similarly to 2012, midges belonging to *Culicoides* *obsoletus*/*scoticus* complex species were the most predominant vector of SBV in Poland. The second important vector species was *C. punctatus*. One positive *C. chiopterus* pool was detected in 2013. Positive nulliparous pools (young females which have not taken any blood meal yet) were detected in each year tested what may suggest independent from ruminant virus circulation in the midge population.

SBV positive pools were detected at the same locations in the consecutive years which shows continuous circulation of the virus despite the intra-seasonal lack of insect activity (possible 'overwintering').

Conclusions: Decline in the percentage of positive pools as well as in the mean load of SBV RNA in 2013-2015 in respect to 2012 suggests low SBV infection rate and possible virus clearance in Poland. This corresponds to the slow increase of seroprevalence observed in farm animals between 2013 and 2014. Therefore, vector SBV monitoring seems useful in order to study the dynamics of the virus spread in the country. SBV overwintering may be connected to the continuous circulation also due to transovarial transmission in the midges.

Emerging Diseases

P02-002-075

Species composition, abundance and new geographical species identification of *Culicoides* biting midges on dairy farms

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Objectives: *Culicoides* biting midges can transmit over 50 arboviruses worldwide including bluetongue virus (BTV) and Schmallenberg virus (SBV). In northern Europe, six species (*C. obsoletus*, *C. scoticus*, *C. dewulfi*, *C. chiopterus*, *C. pulicaris* and *C. punctatus*) are considered to be vectors of veterinary concern. The emergence of BTV and SBV in northern Europe has highlighted the susceptibility of domestic livestock to vector-borne diseases. To date there have been no published studies on these vectors on Irish dairy farms. Hence, the objective of this study was to investigate the species composition and abundance of *Culicoides* on dairy farms in Ireland.

Materials and Methods: Ultraviolet light trapping for *Culicoides* was conducted on 10 dairy farms located in the south of the Republic of Ireland. Each site was sampled fortnightly over 16 weeks (21st July – 5th November 2014). One Onderstepoort Veterinary Institute UV light trap was run overnight (dusk to dawn) in the vicinity of livestock on each farm. Catches were transferred immediately into 80% ethanol for storage. Subsequently, *Culicoides* were morphologically identified to species level. Collection site habitats were characterised using Phase 1 habitat survey technique (Joint Nature Conservation Committee).

Results: A total of 24,190 *Culicoides* (82% female) were collected from 10 farms in 68 successful trap collections. *Culicoides* were found ubiquitously across all sites; however, a large variation in *Culicoides* abundance between sites was observed (range 261-4,641 total *Culicoides*/farm). This may be due to between-farm differences in environmental factors, meteorological conditions or host availability. Collection site habitats were predominately improved grassland which included a mix of semi-natural woodland and hedges. A total of twenty-two different species of *Culicoides* were identified from trap catches. The most abundant species identified were the putative vectors of BTV and SBV (constituting 92.5% of *Culicoides* caught; *C. obsoletus*/*C. scoticus* [37%], *C. dewulfi* [36%], *C. chiopterus* [4.48%], *C. pulicaris* [9%] and *C. punctatus* [6%]) and these were found to be present on all farms. The remaining *Culicoides* species identified (7.5%) were principally the non-vector species *C. achrayi* and *C. festivipennis*. Additionally, within this percentile three non-vector species of *Culicoides* were identified for the first time in Ireland, constituting new records; *C. cameroni*, *C. clastrieri* and *C. manchuriensis*.

Conclusions: The most abundant species identified in this study were the putative vectors of BTV and SBV in northern Europe. These findings are consistent with previous studies carried out on dairy farms in parts of England. The presence and abundance of these *Culicoides* vector species amongst cattle on dairy farms in Ireland and the UK show that disease transmission could re-occur following a new incursion of BTV or SBV into these areas.

Emerging Diseases

P02-002-076

Pronghorn virus, genomic and antigenic characterization and detection in free ranging ungulates in the US.

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Objectives: In addition to the recognized/classic species within the pestivirus genus there are putative species. One of these is pronghorn virus (PHV). PHV was first isolated from an immature, blind pronghorn antelope in the state of Wyoming. The objectives of these studies were to determine level of genetic and antigenic cross reactivity between PHV and other pestivirus species, examine the incidence of exposure to the emerging pestivirus, pronghorn virus (PHV), in wild ungulate populations, and observe clinical presentation in domestic goats and white tail deer following exposure to PHV.

Materials and Methods: Sequencing of the genomic RNA of pronghorn virus from the cell culture supernatant was done using the Ion Torrent and Illumina MiSeq sequencing protocols, as previously described. The 3' end of the genomic RNA was confirmed by 3' RACE using a primer set designed to amplify the 3' terminal 600 bases. Hyperimmune sera against bovine viral diarrhoea virus 1 (BVDV1), BVDV2, border disease virus (BDV) or PHV were produced in goats and cross neutralization studies performed. Serum and whole blood was collected from mule deer, big horn sheep, mountain goat and pronghorn antelope residing in the US state of Nevada. Some tissue samples were collected from wild ungulates found dead in the field. Virus neutralizations were conducted with these sera using BVDV1, BVDV2, BDV and PHV strains. PCR, using pestivirus specific primers, was done on serum, tissue and whole blood samples. The resulting amplicons were sequenced and phylogenetic analysis performed to determine pestivirus species. Domestic goats and white tail deer fawn were exposed to PHV by oral/nasal inoculation and observed for clinical presentation.

Results: Phylogenetic analysis showed that PHV is more distant from the recognized species of pestivirus than the recognized species are to each other. Antigenic comparison, using hyperimmune goat serum, demonstrated that the antigenic cross reactivity between PHV and the recognized pestivirus species is low. PHV was detected in mule deer, big horn sheep and mountain goat samples. Serological surveys of pronghorn antelope populations revealed higher titers against PHV than BVDV1 or BVDV2. Clinical presentation following infection of domestic goats with PHV included mild, short-term pyrexia and a decrease in circulating lymphocytes. Infection of white tailed deer resulted in death in 2 out of 6 animals inoculated. Pre-existing antibodies, against BVDV in deer, did not prevent replication of PHV or decreases in circulating lymphocytes. PHV was not transmitted to pen mates following infection of either goats or white tailed deer.

Conclusions: Despite evidence that pestiviruses circulate in wildlife populations, the impact of exposure and prevalence of these infections are largely unknown. The antigenic and genomic differences between classic pestiviruses and the emerging pestivirus PHV suggest that diagnostics designed to detect classic pestiviruses may fail to detect PHV. PHV appears to be replicating in multiple free ranging ungulate populations in the US and may contribute to immunosuppression and death loss in these populations. Further studies need to be done to determine if PHV can be transmitted from wild ungulates to domestic animals.

Emerging Diseases

P02-002-078

Btvpur Alsap Vaccines Containing Serotype 4 : Proof of Efficacy for The Control of Btv-4 In Cattle

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Objectives: BTVPUR ALSAP is a range of vaccines containing different combinations of Bluetongue virus (BTV) serotypes.

In sheep, efficacy of BTVPUR ALSAP vaccines containing BTV-4 is substantiated through an official claim at EU level of 1 year duration of immunity after single shot vaccination.

In cattle, these vaccines have also demonstrated their efficacy under various field conditions but not all scientific data involved is publicly available.

Hereafter we report the outstanding efficacy of a BTVPUR ALSAP vaccine containing serotype 4 against an experimental challenge, under controlled conditions in cattle.

Materials and Methods: Two groups of 6 BTV naïve calves (1 month old) were maintained in an insect-proof facility throughout the experiment.

Group 1 was not vaccinated and served as control.

Group 2 was vaccinated on D0 and D21 with 1 mL of a BTV-4 vaccine formulated at low antigen content.

Twenty one days after second vaccination (ie D42), Groups A and B underwent a BTV-4 virulent challenge.

After the challenge, all animals were monitored for rectal temperature, clinical signs and viraemia (qRT-PCR) over 28 days.

Serological monitoring was performed at key points during the study.

Results: Maximal rectal temperatures after challenge were significantly lower in the vaccinated group (mean: 39.0°C) as compared to the control group (mean: 39.5°C).

Mean daily clinical score was higher in the control group (G1) than in the vaccinated group (G2) on all except one day. To our knowledge, this is the first report of a BTV-4 challenge model inducing clinical signs in cattle.

Interestingly, the peak of clinical scores corresponded to the peak of viraemia.

Viraemia was detected in all controls (high copy numbers), at all time-points after D49 (inclusive). Conversely, none of the vaccinated animals were ever detected positive.

Conclusions: These results demonstrated that vaccination of cattle with a BTVPUR ALSAP vaccine containing serotype 4 provides full clinical and virological protection against a BTV-4 challenge.

These results are comparable to those obtained over the full range of study protocols used and challenges conducted with other vaccines of the BTVPUR ALSAP range in sheep and in cattle and show that BTVPUR ALSAP may be used for bluetongue disease prevention (clinical protection) and for epidemiological control (virological protection) of BTV.

BTVPUR ALSAP is a registered trademark of Merial.

Emerging Diseases

P02-002-079

Mycoplasma wenyonii outbreak in a Dutch dairy herd: a case reportRuben Tolboom¹; Bert van Niejenhuis²; Menno Holzhauser³¹Boehringer Ingelheim, Alkmaar, ²DAP West-Betuwe, Meteren, ³GD Animal Health, Deventer, Netherlands

Objectives: Some cases with clinical signs related to *Mycoplasma wenyonii* have been reported in the Netherlands. This abstract describes an outbreak of *Mycoplasma wenyonii* in a Dutch dairy herd; the pathogen has been detected and identified using the polymerase chain reaction (PCR)/denaturing gradient gel electrophoresis (DGGE) technique.

Materials and Methods: The dairy farm involved had 115 lactating animals. The herd was on pasture during daytime (min. 6h/day) and housed indoors during night-time. No recent changes in feeding had taken place prior to the clinical signs. The cows were fed indoors a mixture of grass silage and maize. The 21st of September 2015 all cows from an age of three months had been vaccinated with a BVD vaccine. No other herd treatments were administered prior to the clinical symptoms. Young calves until an age of 9 weeks, dry cows and lactating cows are housed in the same shed.

Results: Since 15 September 2015 edema of the rear legs (from hock to claw) and udders was observed in a few lactating cows. Affected udders or legs were not painful. The milk production of these cows decreased significantly (50-60%). No clinical mastitis was seen in the udders with edema. At the peak of the outbreak eight lactating cows showed these clinical symptoms. These cows were all born between April 2012 and March 2013 and thereby in their first lactating. The affected cows had no hyperthermia or pyrexia. The 7th of October 2015 blood samples (vacutainer EDTA) of three cows with clinical signs were taken for investigation. A blood smear was made and analysed using microscopy to detect haematropic bacterial parasites, all samples were found negative (GD Animal Health, The Netherlands). The 3rd of November 2015 six blood samples (vacutainer EDTA) for PCR/DGGE testing were taken from the cows with clinical signs at an earlier stage. All these 6 samples tested positive on *Mycoplasma wenyonii* (APHA Weybridge, United Kingdom). Infected cows with clinical signs didn't receive any treatment. The edema of the legs and udders disappeared slowly within two or three weeks and the milk production increased to a normal level in approximately four weeks.

Conclusions: The udder edema, leg edema and milk loss of the cows on this Dutch farm was caused by *Mycoplasma wenyonii*. This pathogen was diagnosed using a PCR/DGGE test. Most reported cases in the Netherlands are based on clinical signs. Needles, ticks or insects can play a role as vector in spreading *Mycoplasma wenyonii*, in this case the symptoms were present before and after the herd vaccination.

Emerging Diseases

P02-002-080

Schmallenberg virus sero-surveillance: relationships between herd bulk milk and individual cow blood antibody values in exposed dairy herdsÁine Collins^{1,2}; Michael Doherty²; Jim Grant³; Anne Hallinan⁴; Damien Barrett⁵; John F. Mee¹¹Animal and Bioscience Research Department, Teagasc, Moorepark, Cork, ²School of Veterinary Medicine, UCD, Dublin, ³Applied Physics and Statistics, Teagasc, Co. Meath, ⁴Blood Testing Lab, DAFM, Cork, ⁵Sligo Regional Veterinary Lab, DAFM, Sligo, Ireland

Objectives: The bulk milk ELISA (BM-ELISA) is a useful tool to monitor seroprevalence and exposure to infectious pathogens in dairy herds. However, the relationship between bulk milk antibody values and individual animal blood antibody values in dairy cows naturally exposed to Schmallenberg virus (SBV) is unknown. Hence, the objectives of this study were to determine the correlation between individual animal blood ELISA results and bulk milk ELISA results for SBV and to explore the statistical relationships between BM-ELISA results and individual animal blood ELISA results.

Materials and Methods: Blood samples were collected from 4,047 cows and 24 BM samples were collected from 26 Irish dairy herds. Samples were analysed for SBV-specific antibodies using IDVet ELISA testing kits (serum: Schmallenberg virus Competition Multiplex, ID Screen®; test sensitivity 97.6%, test specificity 100%. milk: Schmallenberg virus Milk indirect, ID Screen®; test sensitivity 96.67%, test specificity 99.05%). The mean herd serological data (skewed) were log (natural) transformed and regressed for both sample/positive (S/P) and optical density (OD) values on BM-ELISA results. The statistical relationships between individual animal blood ELISA results and BM-ELISA results were explored by comparing the estimated distribution function curves (EDF-curve) for individual animal blood ELISA results in each herd, pairwise across herds, using the Kolmogorov-Smirnov statistic.

Results: Twenty two herds were BM-ELISA positive (seroprevalence 29.9-100%) and two herds were BM-ELISA negative (seroprevalence 10.5 and 15.8%) indicating that herds with a negative BM-ELISA result may also have been exposed to SBV. There was a significant relationship between the mean herd seroprevalence and BM-ELISA results (R^2 OD serum-BM OD = 0.72, S/P = 0.69, both $P < 0.0001$) indicating that the BM-ELISA is moderately predictive of mean herd seroprevalence. Of the 325 paired EDF-curve comparisons made across the herds, 79 [24%] had similar EDF-curves and of these, 45 [57%] had contrasting BM-ELISA values (S/P% difference $\geq 5\%$) indicating that herds with similar distributions of individual animal blood ELISA results can have contrasting positive BM-ELISA values. In herds with similar positive BM-ELISA results (S/P% difference $\leq 5\%$) 34 paired herd comparisons were identified of which 28 had significantly different EDF-curves ($0.0466 \leq P \leq 0.0001$) suggesting that herds with similar positive BM-ELISA values can have significantly different distributions of individual cow blood ELISA results.

Conclusions: BM-ELISA values are moderately correlated with herd seroprevalence results and may be a useful SBV surveillance tool in dairy herds. However, this novel exploration of the statistical relationships between individual animal blood ELISA results and BM-ELISA results for SBV adopted in this study indicate that these relationships are not as simple as previously thought.

Emerging Diseases

P02-002-081

The detection of severe fever with thrombocytopenia syndrome virus in black goats (*Capra hircus coreanae*)Jun-Gu Kang¹; Jeong-Byoung Chae¹; Young-sun Cho¹; Yun-Kyung Cho¹; Sung-Suck Oh¹; Do-hyeon Yu²; Kyoung-seong Choi³; Hyeon-cheol Kim

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Objectives: Severe fever with thrombocytopenia syndrome virus (SFTSV) is a novel bunyavirus and a causative agent of an emerging disease in China, Japan, and the Republic of Korea (ROK) and is mainly characterized by fever, leukopenia and thrombocytopenia in human. To investigate the prevalence of SFTSV in domestic animals, we collected serum from Korean indigenous goat (*Capra hircus coreanae*) that is the indigenous goat of Korea.

Materials and Methods: Sera were collected from domestic black goats (*Capra hircus coreanae*) in the ROK. Using serum samples, SFTSV-specific genes were amplified by one-step reverse transcription (RT)-PCR and nested PCR and sequenced. The sequence data were analyzed using Chromas and were aligned using CLUSTAL X. To assess the relationships between individual pathogens, a phylogenetic tree was constructed using the neighbor-joining method in MEGA6. Monoclonal antibodies (mAbs) against the recombinant nucleoprotein (NP) of SFTSV were generated to develop a competitive enzyme-linked immunosorbent assay (cELISA) for the detection of antibodies against SFTSV infection in the goats.

Results: A total of 687 serum samples were collected from goats (20 in Gwangwon, 31 in Gyeonggi, 25 in Gyeongnam, 13 in Gyeongbuk, 325 in Jeonnam, 130 in Jeonbuk, 4 in Jeju, 325 in Chungnam, and 24 Chungbuk Province). A total 18/687 (2.6 %) samples were positive for SFTSV using RT-PCR targeting the "S" segment RNA. The size of amplified product was 346 bp. The obtained sequences showed 95.4% ~ 99.7% similarity to KP994441 from feral cat in ROK. A total 40/500 (8.0%) samples were positive for SFTSV using cELISA.

Conclusions: SFTSV readily infects humans with farming-related exposures as well as numerous domestic and wild animals. Molecular and serological data further suggest that the SFTSV circulates widely in the Republic of Korea, Japan and China.

Emerging Diseases

P02-002-082

Post-epidemic Schmallenberg surveillance: prospective bovine serological and Culicoides virological studies

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Objectives: Schmallenberg virus (SBV) was first identified in north-western Europe in 2011. The virus rapidly achieved a pan-European distribution resulting in an epidemic of ruminant abortions, congenital deformities and mild clinical symptoms in adult dairy cattle. There has been limited research investigating whether SBV continues to circulate in previously infected regions. Therefore, the aim of this study was to investigate SBV circulation in dairy herds in 2013 and 2014 following initial exposure to the virus in 2012.

Materials and Methods: Blood samples were collected from 26 dairy herds before and after (spring and autumn) the vector-active period of 2014. Additionally, known SBV *Culicoides* vector species trapped on ten of these farms during the vector active season were analysed for SBV RNA.

In the spring, 5,527 blood samples were collected; 4,047 [73%] cows and 1480 [27%] heifers (12-24 months age). In the autumn 2,444 blood samples were collected; 1,519 [62%] spring-born weanlings (6-8 months old) and a subsample the sero-negative animals identified in the spring (n = 925; 285 cows [12%] and 640 [26%] heifers). Samples were analysed for SBV-specific antibodies using an IDScreen® ELISA kit. Light trapping for *Culicoides* was carried out on ten of the study farms as part of a separate *Culicoides* entomological study (Collins et al, 2015. World Buiatrics Congress). Each site was sampled fortnightly from 21st July to 5th November 2014. One Onderstepoort Veterinary Institute UV light trap was run overnight at each site and catches were transferred into 80% ethanol. *Culicoides* were morphologically identified and pooled according to species and parity status. A total of 138 pools (3,048 *Culicoides*, average 22 pigmented *Culicoides*/pool), from 6 known SBV vector species were tested for SBV RNA. The analysis was based on duplex- real-time reverse transcriptase PCR for the detection of the SBV S segment and the *Culicoides* 18 S gene (*C. obsoletus/scoticus*, *C. chiopterus*, *C. pulicaris*, *C. punctatus*) or the mitochondrial CO1 gene (*C. dewulfi*) as internal control in the in-house optimised real-time PCR system.

Results: In the spring of 2014, animal-level seroprevalence was 61%. Sero-negative animals (38%) were predominantly 2013 spring-born heifers (98%) suggesting they were not exposed to SBV during 2013. Within-herd seroprevalence ranged widely (8.3% to 98%) across herds indicating individual herds had varying risks of new infection in 2014.

In the autumn of 2014, 2,382 (98%) animals were sero-negative and 24 (0.98%) tested positive. Of weanlings, 1,491 [98%] were sero-negative and 9 [0.59%] tested positive. Of the 925 resampled spring sero-negative animals, 891 [96%] animals (268 [29%] cows and 623 [67%] heifers) remained sero-negative and 15 [1.62%] animals (10 cows and 5 heifers) tested positive. The 24 seropositive animals (9 weanlings, 5 heifers and 10 cows) identified in the autumn originated in 14 different herds and the number of seropositive animals in seropositive herds ranged from 1 to 3 animals [0.5-2.8%]. These findings are inconsistent with SBV transmission characteristics. Thus, we suggest that these are false-positive results. Of the 138 *Culicoides* pools examined for SBV RNA one pool (*C. dewulfi*) was weakly positive on initial first test (Ct= 38.11, IC= 19.15) but was negative when re-tested and negative using gel electrophoresis. No other *Culicoides* pools tested positive for SBV RNA suggesting that SBV did not circulate in the *Culicoides* vector during the sampling period.

Conclusions: It is concluded that there was no evidence of SBV circulation in 2013 and 2014 in these Irish herds. However, large populations of naïve weanlings and heifers were identified and these may be at risk of SBV infection in the future should the virus re-emerge; SBV re-emerged during 2014 in Germany. Furthermore, known *Culicoides* arbovirus vectors were definitively identified in these herds suggesting that they are likely have a role in the transmission of SBV and other arboviruses amongst cattle on dairy farms in the future. This is the first study to examine known arbovirus vector species for SBV in Ireland and Great Britain.

Emerging Diseases

P02-002-084

Incursion of SBV in Sweden and situation update in Sweden and Finland

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Objectives: The first indication of the presence of SBV in Sweden was demonstrated by the detection of anti-SBV antibodies in a blood sample collected as a part of surveillance program in small ruminants in autumn 2011. One year later a high seroprevalence of anti-SBV antibodies within the Swedish ruminant population were observed indicating a rapid spread of the infection throughout the country. The objectives of this study were to carry out a molecular characterization of SBV circulating in the Swedish ruminant population from November 2012 to the end of May 2013 and to determine the seroprevalence and evidence of exposure to SBV in 2015.

Materials and Methods: Molecular characterization-Between November 2012 and June 2013, tissue samples were obtained from 328 herds including 169 cattle herds, 124 sheep- 4 goat flocks, 2 alpaca- 1 camel- and 1 bison herds in Sweden. Samples of brain were collected from malformed, aborted or cases of stillbirth fetuses submitted for post mortem examination to the National Veterinary Institute in Sweden. The samples were tested initially for detection of SBV RNA using RT-qPCR. Brain tissue specimens were homogenized using a tissue Homogenizer in TE buffer medium and centrifuged briefly. Homogenization of tissues was performed under biosafety level 3 conditions. RNA was extracted from tissue homogenate using a Magnatrix 8000+ robot (NorDiag AB, Hägersten, Sweden) using the Nordiag Vet Viral NA extraction kit (NorDiag AB, Hägersten, Sweden) according to the manufacturer's instructions. Fifteen SBV positive samples were selected on the base of differences in geographic location, timing and animal [bovine (n=6) and ovine (n=9)] species of origin. PCR assay with M and S segments specific primers was performed to amplify the complete open reading frame of the respective gene.

Serological testing-Serological tests were carried out on sera submitted to the National Veterinary Institute as a part of the health control investigation in 2015. Sera from cattle borne after 2013 collected in southern and central Sweden and Finland were analyzed for presence of SBV-specific IgG antibodies. Sera were screened using a commercially available indirect SBV Antibody Kit, following the instructions of the manufacturer.

Results: SBV was confirmed by RT-qPCR in brain tissues from malformed fetuses obtained from 27 cattle- and 33 sheep herds in Sweden. The phylogenetic analysis of the M gene of the selected number of SBV positive animals from Sweden and the inclusion of sequences from Finland, Denmark and Norway indicated multiple introduction and co-circulation of at least 4 sub-clades of this virus at the time of the study. After very wide spread of SBV during the vector season 2012 and 2013, no typical malformation associated to SBV foetal infection in cattle and sheep were reported in 2014 and 2015. Serological data obtained in animals born after 2013 suggest that SBV did not circulate in Sweden and Finland in 2014 and 2015.

Conclusions: SBV has been considered endemic in Sweden since June 2013 and therefore no further surveillance activities or clinical investigations have been performed. Although SBV is still circulating in several European countries including The Netherlands, Belgium and Germany, no pathological (acute clinical manifestations or an increased number of malformations), virological or serological evidence of current circulation of the virus have been reported in Sweden since 2013. The consequences for future epidemic in Nordic countries will be discussed.

Emerging Diseases

P02-002-085

Schmallenberg virus seroprevalence in domestic ruminants in Poland between 2013 and 2015.

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Objectives: Schmallenberg virus (SBV) affects animal husbandry mostly by causing congenital abnormalities in cattle, sheep and goats. Morbidity and mortality of less than 3% were reported. In adult animals, clinical signs are mild and may pass unnoticed. In Poland, the impact of SBV remains underestimated, since despite that the disease is not registered, the notification of an outbreak would cost a farmer a problem of epizootic restrictions. SBV has spread to Poland in 2012 infecting only some 2% of ruminant population. After SBV overwintering, the virus infected further 30% of animals in 2013.

Materials and Methods: Serum sampling was carried out during national bluetongue virus (BTV) monitoring program. In order to detect an individual SBV seropositive animal, the numbers of samples were calculated using county population statistics estimating 20% seropositivity with 95% confidence level. A total of 14.350 samples from cattle, sheep and goat were collected from July 2013 to May 2015 from 14 out of 16 Polish provinces. Commercial multi-species ID Screen Schmallenberg Virus Competition Test (Montpellier, France) was used to detect anti-SBV nucleoprotein antibodies. Statistical analysis was performed using STATA v. 13.0 software (StataCorp LP, Texas, USA). The prevalences were corrected by the population probability weights (pweights) calculated as follows: 1/(n of sampled animals/N of animals in the province). The associations between the species, gender, age, province, year, domestic ruminant densities per km² and SBV seroprevalence were estimated using χ^2 , Spearman test and odds ratio (OR) calculated by univariate logistic regression. The risk factors of SBV seropositivity were assessed using mixed-effects logistic regression model built accounting for possible clustering and collinearity.

Results: The overall seroprevalences were 35.4%, 58.7% and 29.5% in 2013, 2014 and 2015, respectively. The testing of the samples from 2015 was not finished by the time the abstract was prepared, therefore the possible different outcome should be considered. The association between the province of origin and SBV seroprevalence was significant in 2013 and 2015. The province seroprevalences in 2014 were comparable suggesting even spread of the virus among domestic ruminants. The increase of the percentage of SBV seropositive animals in the provinces (15.3% on average) between 2013 and 2014 was significant especially in the southern provinces. The seroprevalence decline at the province level between 2014 and 2015 was 28.2% on average. In all tested years the highest seroprevalence was found in cattle (57.8%), while it was lower in smaller ruminants (23.3% goats; 39.1% in sheep). The risk factors for the SBV seropositivity were animal age (SBV seroprevalence

decreased with age), gender (females were 0.4 times more likely to be seropositive than male), species (highest seroprevalence in cattle, lower in small ruminants), year of sampling, ruminant densities and province.

Conclusions: The SBV transmitted to Poland in 2012 continued to spread in 2013 and 2014, reaching the peak seroprevalence in 2014. In 2015, the seroprevalence started to decline, however the data is limited. The dependence between SBV seroprevalence and age may be explained by the differences in the exposure time, while the differences among different species may be due to species susceptibility, vector feeding preferences or maintenance. Even SBV geographic distribution and appearance of the virus in the midges collected in different locations scattered in the country may suggest local virus circulation, however at a low level.

Emerging Diseases

P02-002-086

Experimental Infection Of Rabbits With Bovine Herpesvirus 5 (BoHV-5)

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Objectives: Bovine Herpesvirus 5 (BoHV-5) is an alphaherpesvirus associated with neurological disease and meningoencephalitis, usually a fatal viral infection and highly prevalent in South America. Usually, the neurological signs observed in BoHV-5 infections are multifocal deficits and comprise mainly cerebral and brainstem dysfunctions. This study aimed to characterize the clinical, histological and location of the virus in the central nervous system, in rabbits inoculated with a Brazilian strain of BoHV-5 named Mutum, isolated from a five years old cow with spinal cord symptoms, a rarely reported signal for this disease.

Materials and Methods: Six animal inoculations were performed to evaluate the susceptibility of rabbits to a Brazilian strain of BoHV-5. Weanling New Zealand rabbits were intranasally inoculated with 5x10⁷ TCID₅₀ of virus suspension into each nostril and two animals were mock-infected. The rabbits were clinically monitored and blood samples and nasal swabs were collected. Half of the animals was euthanized 5 days post inoculation (pi) and the remaining, in the 12th day. Sections of brain, cerebellum, brainstem, trigeminal ganglia and spinal cord were collected for virological and histological examinations.

Results: The inoculation of weanling rabbits caused neurological disease 50% of infected animals. Until the first six days after inoculation, some animals showed occasional sneezing, beyond nasal and ocular discharge. Other rabbits showed seizures, periods of depression or excitability, tremors crisis (Jackson's seizures), nystagmus and circling, started on the day 6 pi. One of these animals showed a paresis of the hind limbs. Infectious virus was recovered by isolation from cortex, cerebellum, trigeminal ganglia, cervical, thoracic and lumbar spinal cord of infected animals. Viral DNA was detected in the brainstem, cerebellum, cervical spinal cord and thoracic spinal cord of infected animals.

Conclusions: These results confirm the BoHV-5 ability to induce medullary syndrome with ataxia of pelvic members preceding cerebral involvement and highlight the relevance of pointing out these symptoms during clinical evaluation of cattle.

Emerging Diseases

P02-002-087

Towards understanding the increasing threat of digital dermatitis treponemes.

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Objectives: There is now considerable evidence identifying treponemes as the primary causative agents of bovine digital dermatitis (DD), a worldwide infectious lameness of dairy cattle. We have been able to demonstrate these DD treponemes are also implicated in additional, more recently reported infectious lameness in sheep, beef cattle, Wild American Elk and goats. We have also demonstrated treponeme contribution to bovine non-healing hoof lesions and hock lesions.

This study set out to investigate the genetic relationships of DD treponemes from a range of hosts using a multi locus sequence typing (MLST) analysis.

Materials and Methods: The cultivable DD treponemes have previously been classified into three phylogroups, based on 16S rRNA gene similarity to human treponeme species and by which they are frequently referred as '-like': Treponema medium-like, Treponema phagedenis-like and Treponema denticola-like, with the latter recently taxonomically designated as Treponema pedis.

The fastidious nature of these important spirochetes previously precluded studies investigating within phylogroup genetic diversity. An archive of treponemes isolated by the authors here enables MLST to quantify diversity and potential transmission events of DD treponemes. Isolates (n=118) were obtained from different animal hosts (including dairy and beef cattle, sheep, goats and Wild American Elk) representative of nine countries and three continents. Implementing the MLST for these bacteria included PCR and DNA sequencing of seven different housekeeping genes from all isolates, and subsequently comparing them using a range of phylogenetic analyses.

Results: The seven different housekeeping genes were sequenced for each isolate and demonstrated low level diversity between strains for each phylogroup. The allelic profiling allowed the typing of strains from the variety of hosts and showed that all animals can be infected with very similar bacteria, with some animals identified as likely infected with the same bacteria. Human treponemes, whilst displaying novel allelic profiles, still only differed by a small number of nucleotides substitutions when compared with from animal isolates.

Bacterial sequence types (ST) were shared across worldwide geographic regions and different hosts in several cases. Other ST's appeared to transmit around a farm, infecting multiple animals on one farm, whereas others infect multiple hosts in similar geographical areas, suggesting between host transmissions. Of the three phylogroups T. pedis was the most variable, with lower levels of diversity seen in the other two phylogroups.

Conclusions: Human and animal treponeme similarity suggests these phylogroups may have been transmitted between man and animal at some point. It might be considered DD treponemes are not readily differentiated by MLST. However, that identical STs were shared between isolates between animals on the same farm and from different host species in similar geographic regions suggests that bacteria can spread between animals on a farm, and between host species. The expanding host and tissue range of DD treponemes requires vigilance given the substantial animal welfare issues, lost productivity and costs towards treatment and control of associated diseases.

Emerging Diseases

P02-002-088

Schmallenbergvirus in Sheep: Persistence of Antibodies after Infection

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Objectives: After the appearance of Schmallenbergvirus various studies were performed. But few studies were made about the persistence of antibodies after infection. Especially in sheep there are nearly no data on duration of immunity. The data in cattle suggest that immunity last for at least one year following natural infection.

Materials and Methods: In this study sheep from two herds were included, one herd in northern Bavaria with 400 animals and one in the south with 800 ewes. Several blood samples of individual animals of different ages were taken. 106 sheep from the northern herd and 90 from the southern herd were followed individually. The samples were analyzed with the ELISA Test "Schmallenberg ID Screening". The first herd was tested three times between January 2013 and February 2014. In the second herd six samples were taken between February 2012 and May 2014.

Results: The northern herd was included later in the study, with already seroconverted animals. But 62 sheep from the 106 animals were seronegative during the whole study and 15 animals were seropositive in every sample. Six animals were positive in two samples, 7 only in the first sample and afterwards they were negative.

The samples in the southern herd were first taken in February 2012, were nearly all 55 animals were seronegative, only one animal was positive and stayed positive in all samples during the two years. 41 sheep seroconverted between May 2012 and August 2013 and stayed seropositive until April 2014. In August 2013 20 yearlings were tested, all negative in the four following samples. Also the 15 new yearlings of Mai 2014 were negative.

Conclusions: These samples showed that antibodies against Schmallenbergvirus can persist for minimum two years following natural infection in sheep. Individual animals were tested and individual durations of antibodies could be shown, with variations from several months to two years. It can only be estimated that these antibodies are protective against illness, because in the year 2014 there were very few cases of Schmallenbergvirus in Bavaria. The herds are followed up during the next months.

Emerging Diseases

P02-002-089

Are potentially toxigenic Clostridium spp. (especially Clostridium botulinum) a significant factor for chronic health problems in dairy herds?

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Objectives: In recent years, unusual herd health problems in dairy cattle have been reported by veterinarians and farmers across Germany that manifested as chronic suffering with severely decreased milk yield and significant losses up to complete abandonment of herds. According to reports, Clostridium botulinum and/ or its toxins and/ or antibodies were detected in affected animals. The authors of these reports named this previously unknown form of botulism "chronic/ visceral botulism", and – in contrast to classic botulism – thought it to be caused by toxicoinfection.

Materials and Methods: In a case-control study across Bavaria, Germany, herds in the case group (n=21) had to meet at least three of the following criteria: a decrease in milk production of more than 15%, more than 5% of animals euthanized or dead within the last twelve months, a culling rate higher than 35%, recumbency in more than 10% of animals, and an increased disease rate. Herds in the control group (n=10) were not allowed to meet any of these criteria. A total of 1720 dairy cows were included in the present study of which 284 (case and control cows) were examined further. After passing a standardized clinical examination, the animals were sampled for bacteriological, parasitological, serological and hematological examination. Questionnaires were used for additional data collection and covered specific fields within each farming enterprise.

Results: Analysis of fecal and rumen fluid by real-time PCR revealed, that Clostridium spp. were present on 74.2% of the dairy farms evaluated. There was no difference in the frequency of detection between the case and the control group or between the case and control cows on each farm. Species of Clostridium were distributed as follows: Clostridium perfringens 72%, Clostridium novyi 24%, Clostridium botulinum 3%, and Clostridium haemolyticum 1%. Serum antibodies against BoNT-C1 and -D were detected in 78.2% of the farms with no statistically significant difference between the case and control group. However there was a statistically significant difference between the cows within the case group (84.6% positive - control cows versus 70.2% - positive case cows). Cows in the case group were significantly dirtier, skin lesions on bony prominences of the body proximal to the carpus/ tarsus were significantly more often detected and prevalence and severity of lameness were significantly higher than in the control group.

Conclusions: The data analysis showed a significantly lower hygiene and a significantly higher prevalence of lameness and skin lesions in the case group than in the control group. However, there was no correlation found between any of these factors and the frequency of detection of Clostridium Species.

Emerging Diseases

P02-002-090

Emerging Stealth Assault by Mycoplasma bovis in Sucklers in Pakistan

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Objectives: *Mycoplasma bovis* is one of the components of the bovine respiratory disease complex (BRD), which is mainly characterized by

pneumonia. The BRD complex inflicts considerable financial losses worldwide. Variable prevalence of *Mycoplasma bovis* infection has been reported in small and large ruminants around the world. However, no information is available regarding pathophysiology of this microorganism in dairy cattle of Punjab, Pakistan. Therefore, present study was conducted initially to investigate this organism hideout in a local dairy herd.

Materials and Methods: The present study was conducted to investigate the pathology of *Mycoplasma bovis* infection in local, 2-4 week-old *Bos indicus* dairy calves. In a herd of 50 calves, 15 (30%) became sick and 5 (10%) died soon after transportation which were subjected to necropsy. PPLO broth and agar plates were inoculated with nasal swabs from clinically sick calves and pus samples from abscesses. These were incubated at 37 °C with 5% CO₂ for 7-10 days and monitored for fried egg colonies. Furthermore, molecular identification of *Mycoplasma bovis* was done to confirm the occurrence of *Mycoplasma bovis* in dairy calves.

Results: Clinical signs included respiratory distress, lameness and swollen joints. During postmortem examination, severe suppurative polyarthritis and fibrinosuppurative tenosynovitis were observed. The knee and stifle joints were most severely affected. Otitis media was a common feature in all necropsied calves. Multiple abscesses with caseous exudate were present in cranioventral lobes whereas dry necrotic cavities in diaphragmatic lobes of the lungs. Microscopically, synovial cavities and membranes of the joints exhibited multiple foci of central liquefactive necrosis surrounded by lymphocytes, plasma cells and macrophages, with peripheral fibroplasia. Immature abscesses were exhibiting infiltration of neutrophils. In the lungs, multiple well circumscribed abscesses were minimally calcified. *Mycoplasma bovis* infection was confirmed by PCR.

Conclusions: In conclusion the finding of the present study indicated that *Mycoplasma bovis* infection is a serious threat to the dairy animals in Pakistan. In addition to any economic consequences associated *Mycoplasma bovis* must be considered significantly important and further studies are required to reduce the economic losses in dairy cattle of Punjab, Pakistan.

Emerging Diseases

P02-002-091

An evaluation of the potential of text analytics to a Veterinary Pathology and Surveillance Service

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Objectives: Animal health surveillance should constantly reinvent both the sources of data and the modes used to analyse it. Pathology is a versatile discipline well positioned to detect emerging and changing animal disease. Traditionally, pathology services categorise case-loads using frameworks and classifications agreed a priori. However modern IT systems enable very large datasets to be iteratively re-analysed in real time. This report objectively analyses a very large free-text veterinary pathology database, to test the advantages and added value obtained by treating such a highly technical source simply as an extremely rich text mine.

Materials and Methods: The period chosen for analysis was 24 months: 1st Jan 2010 to 31st Dec 2013. The Irish Veterinary Laboratory Service LIMS system database was interrogated on 22/02/15 and an extract obtained. The dataset consisted of 75, 047 pathology reports (clinical,

post-mortem and histology) with a total of 2,165,565 words covering submissions from all six Regional Veterinary Laboratories (RVL).

The dataset was filtered to retain only submissions related to bovine animals and common link words such as “the, and, of, with, a”, etc, were removed from further inclusion.

The analysis tested both submissions and individual words in terms of geo-location, month, frequency, etc and also examined cross-links with other clinical submission data available.

Results: The average number of words per submission was 29.5, with relatively little seasonal but some minor RVL effects detected.

The commonest pathology terms were pneumonia, congestion, oedema, diffuse and enteritis with frequencies of 5984, 5693, 5229, 4233 and 4198 respectively. In contrast, the words infarct, dermatitis, cirrho, bullous and malacia had frequencies of 82, 64, 61, 58 and 52 respectively. The histological partial terms infiltrat, monocy, macrophag, lymphocy, eosinoph and basoph recorded frequencies of 409, 271, 249, 229, 137 and 31 respectively.

The words enteritis and pneumonia had marked frequency peaks between February and May each year while hepatic and nephrosis recorded no seasonality. The word Dictyocaulus had a clear frequency peak between July and November, while Fasciola had a pronounced trough in June and July. Further analysis demonstrated distinct patterns of word frequency associated with particular cattle age groups and particular months with graphical demonstration most effective.

Conclusions: Pathology services produce unique flows of expert opinion and highly detailed intelligence on disease diagnosis and effects which are ideally suited to text mining techniques. Such data sources are independent of diagnostic techniques and could operate near real-time or be automated to trend accessibly based on frequency. A text analytics approach requires no prejudice, maximises flexibility and has significant training potential. Best of all, it allows pathologists to concentrate on pathology without the overhead of classification.

Emerging Diseases

P02-002-092

Genotypic Variation Of Coxiella Burnetii In South-West Dairy Farms In The Uk.

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Objectives: Over the last 10 years research into the zoonotic disease Q Fever, and the causative bacteria *Coxiella burnetii*, has increased considerably. This increase is mainly as a result of the large-scale outbreak in The Netherlands from 2007 to 2009. Human infections are primarily due to exposure to infected ruminants, particularly around parturition. This poses a threat to farm workers and veterinarians. Whilst infected goats have been attributed as the primary cause of the Dutch outbreak, dairy cattle are still considered a potential reservoir for the spread of this disease.

Materials and Methods: Through bulk tank milk analysis, using qPCR, *C. burnetii* has been found in South West dairy farms at a herd prevalence of 69.7% (108/155). We wanted to investigate the molecular epidemiology of these samples by determining if the genotype was related to *C. burnetii* found in Europe.

Multiple Locus Variable number tandem repeat (VNTR) Analysis (MLVA) has been shown to be a viable genotyping assay for *C. burnetii*, as devised by Arricau-Bouvery et. al, although some modification was required to detect organisms from Bulk Tank Milk (BTM) samples. We aimed to utilise this assay for the positive BTM samples, by selecting a sub-set of primers which are able to differentiate UK strains of the bacterium.

The size of the VNTR alleles is converted to units, with clustering analysis on these units. Clustering analysis is by Unweighted Pair Group Method using Arithmetic averages (UPGMA).

Results: Initial analysis has led to the possibility of multiple genotypes being identified in single BTM samples. For a number of samples, more than 1 PCR product was visualised following gel electrophoresis. These additional PCR products were larger than from other samples, indicating that additional tandem repeats were present. Full MLVA analysis utilising capillary gel electrophoresis to determine exact amplicon length is still ongoing. These genotypes are being compared to those found in the rest of Europe, in both dairy cattle and small ruminants from a collaborative MLVA dataset. This

Conclusions: As this investigation is still ongoing, it would be premature to draw any confident conclusions. Once completed, knowledge of the MLVA genotypes present in the UK will be of interest to European peers researching this bacteria. To our knowledge, this only the 2nd time *C. burnetii* has been genotyped in the UK and the first time in UK dairy cattle.

Emerging Diseases

P02-002-093

Our experience with Lumpy Skin Disease epidemic in Greece in 2015

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Objectives: Lumpy Skin Disease (LSD) is an acute viral disease of cattle that is endemic in most of Africa and Middle East. In August 2015, LSD was first time spread to Greece with the epidemic starting from the border with Turkey. This is a report of the infection epidemic in respond to the Greek State received measures; also includes our clinical experience with a limited number of animals diseased. At the time of writing this abstract, the epidemic event is continuing.

Materials and Methods: In the suspected cases, the disease was confirmed by real-time PCR in heparinized total blood. Analysis was carried out in the Athens Veterinary Center (National laboratory).

In face of the epidemic, the Greek State implemented an extensive stamping-out policy, zoning and movement control inside the country. Emergency vaccination campaign of all cattle against LSD was progressively applied in the regions of the outbreaks. The vaccination was completed in the entire Evros regional unit up the middle October 2015; while the vaccination is still in progress (end of November 2015) in the entire Xanthi, Rodopi and Kavala Regional Units.

Results: Up to the middle of November 2015, one hundred four (104) infected farms had been confirmed and the infection had been spread southern to the central Macedonia, in the Regional Units of Chalkidiki and Thessaloniki, and in the Limnos Island. In the infected farms a number of

474 cattle had been found clinically diseased and 5,161 heads had been destroyed during the implementation of the stamping-out policy.

A number of the cattle clinically affected brought to our attention. Clinical signs initially included lachrymation, fever (>40.5 °C), loss of appetite, and disinclination to move. Approximately one week after the initiation of the fever, nodules were appeared at all over the body and swelling of the superficial lymph nodes. The diameter of the nodules was 0.5 to 1.5 cm, size relatively small in comparison with the description of the literature (2.0 to 5.0 cm). At the time of skin nodules appearance, soft yellow-grey nodules and ulcers were occurred in the mucous membranes of the mouth, nose, respiratory tract and the genital tract. Salivation, nasal discharge and corneal opacity were frequently seen. All cases were destroyed within the first days of nodules appearance and stages of nodules that exude serum, break away, form scabs and excoriate were not seen.

Conclusions: The stamping out policy in combination with vaccination was proved a useful tool for the control of the infection. However in the case of the described epidemic a delay of the vaccine application was noted due to unavailability of the vaccines. This delay of vaccination contributed to the disease spread. Other causes of disease spread were the high temperatures till the end of November 2015 and the illegal livestock moves.

Epidemiology

P02-002-238

IBR and BVD prevalence in President Epitácio and Mirante of Paranapanema settlements, São Paulo, Brazil.

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Objectives: The purpose of this research is conducted clinical assessment and establish the regional prevalence of IBR and BVD, by studying the dairy cattle herds of micro settlements of President Epitacio and Mirante de Paranapanema cities.

Materials and Methods: The settlement belonging to the region of President Epitacio and Mirante do Paranapanema cities that have approximately a total of 917 settlers and 17,934 cows distributed among them. Milk production of these settlers come to 14,980,919 liters of milk, the main economic activity of this regions. 1. -To select the number of animals and properties to our collection to take a prevalence of the presence of IBR and BVD, 50% were choosed animals and 10% in the properties, with a confidence level of 95% and a specificity and a sensitivity of 80% , in properties with 1 to 200 animals. Obtained this calculation were selected 41 properties and 4 cows per property. 2. – On each cow became an clinical examination of the genital system through an obstetric examination (rectal palpation) and gynecological examination (vulva external inspection, vaginoscopic exam to observe the format, color and opening of the cervix) by Grunert, 1994 methods. 3. - approximately 10 ml of blood were collected and was separated non-haemolysed serum. Serum was stored in a cooler at a temperature of about 4 ° C until their arrival at the laboratory. The serum was tested with the serum neutralization techniques for BVD and IBR like described by Pituco et al. 1994 and 1996.

Results: For a better understanding of our results, we used the statistics program Minitab 2015. Of the 168 samples collected in Mirante do Paranapanema and Presidente Epitácio settlements, São Paulo, Brazil, 116 were positive in the serum neutralization test resulted in one prevalence of 69,04% for IBR. These same 168 colected samples, 97 were positive in the serum neutralization test, resulted in a prevalence of 57.73% for BVD. The results show that the both regions studied have presence of IBR and BVD virus and we found abortion in this same region in animals who not receive vaccine for control these diseases.

Conclusions: We can conclude that the prevalence of IBR and BVD in President Epitácio and Mirante do Paranapanema settlements is high, 69,04% and 57,73%. This results was the first established for the State of São Paulo settlements and we have to achieve this problem and helping the settlers to receive more information about these diseases and how they can eliminated this problem in their herds.

Epidemiology

P03-003-067

An online calculator for assisting in the design of herd-level prevalence surveys whilst taking into account test imperfections

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Objectives: Most practitioners in epidemiology will be familiar with the fact that calculating sample sizes for surveys is often an iterative process due to there being more than one adjustable variable. This is especially so in veterinary epidemiology of livestock because we may have more than one stratum at which to sample - namely animals within herds and sampling the herds themselves. Our online calculator adopts a relatively simple method (Humphry et al., 2004) for the question of determining the number of animals and the number of herds to test in order to achieve a desired confidence about a measured herd level prevalence.

Materials and Methods: For this calculation there is a trade-off between the number of animals in the herd and the number of herds to be tested. This trade-off is hard to explore without testing various different scenarios. Our calculator presents a suite of scenarios via simple 2-D graphs so as to assist the practitioner in “exploring the options” more holistically than is done using the more conventional style of calculator which gives a single result for a single scenario. The calculator was built using the statistical software R (CRAN, 2015) and the Shiny server software produced by RStudio (RStudio, 2015).

Results: Our poster briefly describes the method in the black box alongside screen shots of the calculator with accompanying interpretation in order to demonstrate its utility.

The calculator itself can be accessed for free and the output can be downloaded as a csv file for those wishing to say their analysis. The graphical output contains additional information for users allowing us to display more than just two output variables. Users can change parameters and so get results for their own sample size questions.

Conclusions: The calculator is available here – <https://epidemiology.sruc.ac.uk/apps/>. It should be useful to any practitioner seeking to make an informed decision on sample sizes within and between flocks/herds when designing a herd prevalence survey using an imperfect test.

References: CRAN, 2015 <https://cran.r-project.org/>. Humphry et al., 2004. P.V.M. 65: 173-188

RStudio, 2015 <https://www.rstudio.com/products/shiny/shiny-server/>

Epidemiology

P03-003-068

Identification, Antimicrobial Resistance And Rapd Profiles Of Staphylococcus Aureus From Bovine Mastitis In Ningxia, China

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Objectives: The objective of this study was to isolate and identify Staphylococcus aureus (S. aureus) from bovine mastitis in Ningxia, China, to evaluate the antimicrobial resistance, and also to investigate genotypes of the isolates.

Materials and Methods: A total of 327 milk samples were collected from bovine mastitis cases, streaked onto culture media, and then the

isolates were identified by microbiological and biochemical methods. 16S rRNA sequence of *S. aureus* was used for the isolates identification of the polymerase chain reaction (PCR). The antimicrobial susceptibility tests of the isolates were performed by disk-diffusion agar method and a total of 24 antimicrobial agents were used to evaluate the antimicrobial resistance. The genotypes of isolates were investigated by randomly amplified polymorphic DNA (RAPD) and Bio-Numerics analysis software was used to do cluster analysis of the isolates.

Results: The results showed that a total of 35 isolates coming from 10 dairy farms were found to be positive for *S. aureus* by morphological and biochemical methods, in which 5 isolates were from clinical mastitis and 30 isolates came from subclinical mastitis. Furthermore, the results of 16S rRNA suggested that the target fragment of 1551 bp was amplified from all of the 35 isolates respectively. Therefore, the biochemical test results of *S. aureus* were entirely consistent with the molecular identification results of 16S rRNA. The *S. aureus* isolates displayed differences in the antimicrobial resistance. The isolates were resistant to penicillin G, ampicillin, erythromycin, azithromycin, tylosin, streptomycin, clindamycin, lincomycin, doxycycline, sulfadiazine, and sulfamethoxazole. However, all of them were susceptible to amoxicillin, cephalothin, cefradine, amikacin, neomycin, florfenicol, ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin. The RAPD assay showed that 35 *S. aureus* isolates were divided into 6 genotypes, in which genotype I contained three isolates, genotype II included one isolate, genotype III was composed of 13 isolates, genotype IV had two isolates, genotype V consisted of 4 isolates, and genotype VI had 12 isolates. The most prevalent genotypes of *S. aureus* in Ningxia area were found to be genotype III and genotype VI. There was significant variation in the genotypes of *S. aureus* isolates from various dairy farms.

Conclusions: It was concluded that 35 *S. aureus* isolates were confirmed and divided into 6 genotypes on the basis of RAPD, and the data showed that genotype III and genotype VI were more prevalent in Ningxia area of China. It came to the conclusion in the current study that *S. aureus* showed different antimicrobial susceptibility and resistance against various antibiotics. Therefore, it is recommended to vaccinate the animal against the genotype III and VI of the *S. aureus* for control and prevention of bovine mastitis.

Comments: This research was financially supported by Ningxia Science and Technology Support Project (No. 2013).

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Epidemiology

P03-003-069

National plan for tuberculosis eradication. Case study in a district of northern Sardinia

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Objectives: After few cases of bovine tuberculosis (TB) caused by *Mycobacterium bovis* (Mb) recorded in 1998 in Sardinia, in 2007 the Local Health Veterinarians (ASL) of the Province of Sassari revealed many TB outbreaks, with an increased incidence in the area of Goceano, a district which includes 11 municipalities. The objective of this study is to present the result of the TB plan in Goceano since 2007.

Materials and Methods: The area Goceano is approximately 482 km² with about 10.000 cattle bred in 511 farms. A number of risk factors were identified: issues in the system of cattle identification; fragmentation of farms; common use of public pastures resulting in promiscuity among cattle of different farms and between cattle and wild boars; limits of TB testing with IDT-bovis. To reduce the risks a number of solutions were adopted; 1) the integration of intra-rumen electronic transponder with the ear tags commonly used. 2) geo-referencing of common grassland and farm premises using dedicated software which allowed traceability of all animal movements. The Geographic Information System (GIS) software provided daily updated maps showing the actual number and identification of bovines. 3) Improving the specificity of the TB testing with IDT-bovis, in addition, Gamma-Interferon was used in all cattle over the age of 6 months. TB testing was intensified with tests every six months. All cattle IDT-positive or borderline at the Gamma-Interferon were slaughtered (about 1000 animals). The carcasses were inspected, lesions and lymph nodes sent to the reference laboratory (IZS of Sardinia). All cattle leaving farms were TB tested. Wild pigs were used as bio-indicators of the state of contamination of the territory from *M. bovis*.

Results: From 2007 to 2012, the local health veterinarians highlighted and denounced 75 TB (*M. bovis*) outbreaks in the province of Sassari, 90% of those were in the district of Goceano. All cattle underwent TB testing, with corrective actions taken promptly, including; tightening of controls by vets, using additional diagnostic tests, employing an innovative information system that made possible to identify, track and trace effectively cattle, therefore improving the effectiveness and efficiency of the veterinary service.

The prevalence of outbreaks, in the province, has steadily declined, from 2.4% in 2007 to 0.10% in 2012. Outbreaks gradually withdrawn until December of 2010 when only 3 were notified. In 2013 and 2014 cattle were TB tested only before movements, still obtaining a reliable health status of herds. In 2015 all cattle underwent TB testing no outbreaks were notified.

Conclusions: Corrective actions have allowed the recovery of *M. bovis* in the territory of Goceano. The limits of the test IDT imposes strict health measures which remain in the province. The recent Ministerial Decree of 28 May 2015 reiterated the importance of the traceability of all bovines movements allowed by the electronic identification and the need for effective and rigorous health checks both on farms and in slaughterhouses. It is vital to continue to monitor the wildlife as a bio-indicator of the contamination of a territory by *M. bovis*.

Epidemiology

P03-003-070

Johne's disease and Bovine Viral Diarrhea bulk milk surveillance in the Western U.S. - regional prevalence and positive farm characteristics

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Objectives: Testing for Johne's disease (JD) and Bovine Viral Diarrhea virus (BVD) in dairy herds in Utah and southern Idaho was performed with the major objective of estimating regional dairy herd-level prevalence. Secondary objectives were: for JD, evaluation of changes in individual herd test status compared with previous regional surveillance; estimation

of sensitivity of a single bulk tank milk test for detection of each disease; evaluation of dairy herd characteristics and farm management practices for farms test-positive for either or both diseases.

Materials and Methods: Field personnel from the 2 main milk buyers in the study area collected signed participation forms from producers. Duplicate milk samples were collected by milk haulers at 3-4 day intervals on each of 5 dates from each bulk tank on participating farms. Samples were coded for anonymity, frozen (-20° C), and one sample was shipped to Antel BioSystems for Mycobacterium avium subsp. paratuberculosis, the JD agent, testing with both ELISA and quantitative real-time PCR. The other paired sample was shipped to The Dairy Authority for BVD testing with real-time reverse transcription PCR. Because the tests have nearly 100% specificity, true positive herd status for each disease was defined by finding at least one tank sample test-positive. Sensitivity was defined as the total number of positive test results for each agent divided by the total number of tests performed on all bulk milk samples from the herds true positive for that agent.

Farms positive for JD or BVD were contacted through the milk buyer using their number code and offered a follow up program. If they agreed, their identity was released to the investigators. A questionnaire developed and used for previous projects was slightly modified to evaluate farm management practices and characteristics associated with JD and BVD. Initially, 6 farm visits were made by all 4 field investigators (DW, KR, CW, JB) together to standardize observations and interview methods. Remaining farm visits were made in pairs or by single evaluators. Surveillance results were compiled and analyzed (Microsoft Excel).

Results: Permissions were signed by 151/209 (72%) of producers. Milks (n = 1822) were collected from all tanks. 67 herds (44%) were either JD+ or BVD+: JD 58 (38%), BVD 14 (9%), 5 both. Proportion of positive herds' total samples positive ranged from: JD 5% - 100%; BVD 6% - 100%.

The same region was surveyed previously for JD. Of 67 farms JD+ 4 yr earlier, 25 did not participate or dispersed. 42 were retested: 28 (67%) remained JD+ and 14 (33%) tested negative. Of 103 farms JD-negative earlier, 65 were re-tested and 13 (20%) became newly +. Of farms JD tested for the first time, 17/44 (39%) tested +. Sensitivity (+ tank results/total number of tank samples tested from positive farms) for a single bulk milk sample test for the disease was: JD 284/528 = 54%; BVD 41/117 = 35%.

Visits were made to 22 farms, 21 JD+, one BVD+, one both. Means: 778 milking cows (52 - 6523), 305d milk 9240 kg (6773 - 11,509), SCC mean previous 6 mo 175,000/ml (94,000 - 300,000). Holsteins (95%) and Jerseys (5%) were main breeds. All parlor milking, 6 - 160 milking units. Freestalls for milking cows 91%, dry lots for dry cows 55%. JD+ farms had seen: cows becoming thin but eating 81%; cows diarrhea progress to death 52%. Both BVD+ farms had seen abortions. All 22 farms vaccinated vs. BVD.

When replacements last purchased (41% ≤ 1 yr ago): no testing or segregation 64%, vaccinate new animals 27%, segregate 14%, testing 11% (none for BVD or JD). Calves fed only individual colostrum and pasteurized milk 27%. No producers would segregate JD+ cows or have a separate calving area for JD+ cows or BVD+ cows.

Conclusions: Herd-level prevalence of JD and BVD were similar to previous reports. Most herds JD-positive 4 yr earlier remained so. Herds tested for the first time had the same prevalence of JD as herds tested for the first time 4 yr earlier. Positive herds' size, milk production and quality were above regional average. Most farms had seen disease signs.

Biosecurity was lacking. Most farms did not segregate or screen new additions for disease. Other recommended control practices were also poorly adopted. However, farms that participated in the earlier surveillance program and were JD-negative were less likely to become newly test-positive for JD.

Comments: If you do not think this fits in Epidemiology, please consider Herd Health Management: Dairy or Paratuberculosis as alternatives.

Epidemiology

P03-003-071

Endemic infection with *Coxiella burnetii* in dairy cow herds follows a defined cycle

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Objectives: Q fever is caused by the zoonotic pathogen *Coxiella burnetii* (Cb). Serological diagnosis in human medicine relies on two antigens: Phase I (PhI) and Phase II (PhII). PhII-antibodies are detected early after infection, the development of high-level PhI-titers is indicative of a chronic course of infection. Individual milk samples from dairy cow herds were analyzed for phase-specific antibodies and age-related antibody profiles were assessed to get insights into the epidemiological dynamics of infection.

Materials and Methods: 2880 individual milk samples from 45 dairy cow herds (cows per herd: 64+/-23) were collected. PhI- and PhII-specific antibody titers were assessed and, provided the farmer agreed, the samples tested for Cb by quantitative PCR. In one chronically infected herd all animals (cows, heifers and calves) were additionally tested in a Cb-specific interferon-γ-recall assay (IFN-γ-RA). Finally, the phase-specific serological data from the previous year were available for another chronically infected herd and were compared with the current profile.

The age of each animal was recorded as year and month of birth (YYMM). For each herd a titer-over-age-profile was generated, by sorting the animals by age and depicting the associated titers as well as the moving average of titers for 5 animals.

Results: Three distinct titer profiles were observed at herd level, which we defined as (1) silent, (2) acute and (3) chronic profile. Prototypes are described as follows:

- (1) The silent profile was characterized by the absence of antibodies in cows younger than 3 years. A peak of PhI- and PhII-titers was frequently observed among a distinct age-group of older cows, probably the result of an infection in the past. Remarkably, older cows beyond this peak showed again a lower level of titers and a dominance of PhI/PhII⁺-pattern. Absence of antibodies in the whole herd is an exceptional case, which was rarely observed.
- (2) The acute profile was associated with a dominance of PhI/PhII⁺-pattern in 1st-lactation cows. PhI-titers are rarely observed, if detected they were restricted to defined age-groups.
- (3) The chronic profile was distinguished from the acute one by an abundance of PhI⁺-cows in almost all age-groups (>3 years).

In order to illuminate the aspect of cellular immunity, we analyzed blood samples from all animals for antibodies and IFN-γ. Heifers and calves (>6 months) were antibody-negative but showed a strong IFN-γ-reactivity. Introduction of such immune heifers most likely limits ongoing endemic infection.

The transition from acute to chronic was demonstrated in another farm, in which an acute profile had been observed in spring 2014. Eighteen months later the acute profile changed to a chronic one.

PhI-titers ≥ 100 along with qPCR-positive results (both in milk) are indicating a chronic shedding of Cb in milk. Such cows are detected even in herds with silent profiles.

Conclusions: Our data promote the idea of a defined Q fever cycle in dairy cow herds. The shift from the chronic into the silent stage relies on immunity of the young stock probably acquired in early calf hood. After some time in the silent stage susceptible calves and heifers are born and raised. A growing number of such susceptible cows increase the risk of an acute infection. Cows shedding Cb chronically in milk might be the source for reactivation of acute infection. Primary infection of susceptible cows results in PhII-titers; PhI-titers are generated later and some of these animals are going to establish a chronic shedding of Cb in milk.

Comments: This study was financially supported by the Free State of Bavaria and the Bavarian Joint Founding Scheme for the Control and Eradication of contagious Livestock Diseases.

Epidemiology

P03-003-072

First evidence of Schmallenberg virus introduction in Portugal, 2014

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Objectives: Since the emergence of Schmallenberg virus (SBV) in 2011 this virus has spread over Europe, inclusively reaching Spain in March 2012. Despite the close proximity to the Portuguese border, until date there are no reports of SBV infection in Portugal. Hence, a serosurvey was set up to estimate the presence of IgG antibodies against SBV (IgG anti-SBV) in sheep of Portugal that could allow to infer about the circulation of this virus in the Portuguese territory

Materials and Methods: The sample size to estimate the occurrence of IgG anti-SBV in sheep of Portugal was calculated assuming SBV seroprevalence of 50% (yielding the highest possible sample size), confidence in the estimate of 95%, maximum allowable error in the prevalence of 3%, and population size of 1,852,940 sheep (official animal census data). A stratified random sampling design was set up with the 5 regions of Continental Portugal as a stratification level, according to the Nomenclature of Units for Territorial Statistics level II (North, Centre, Lisboa and Vale do Tejo, Alentejo and Algarve). Blood was collected from November to December 2014 from female sheep with 6 months to 10 years of age (N = 1,068) randomly selected upon the moment of the official brucellosis control. Sera were immediately separated and tested for the presence of IgG anti-SBV using an enzyme linked immunosorbent assay (ID Screen® Schmallenberg virus indirect, IDvet Innovative Diagnostics) according to the manufacturers' instructions.

Results: Of the 1,068 sheep serum samples tested for IgG anti-SBV, 137 were found positive, giving an overall SBV seroprevalence of 12.8% (95% CI 11.0%–15.0%). However IgG anti-SBV prevalence showed to be different in the different regions of the country, being 8.1% (95% CI 4.8%–13.3%) in the North of Portugal (13/161), 14.2% (95% CI 10.3%–19.2%) in the Centre (N = 34/240), 31.6% (95% CI 15.4%–54%) in Lisboa and Vale do Tejo (N = 6/19), 12.7% (95% CI 10.3%–15.5%) in Alentejo (79/624) and 20.8% (95% CI 9.2%–40.5%) in Algarve (N = 5/24).

Conclusions: This is the first nation-wide serosurvey providing evidence for Schmallenberg virus introduction in Portugal - The SBV seroprevalence in sheep showed to be lower in the North of Portugal than in the remaining parts of the country. We hypothesize that this could indicate that SBV was first introduced into the central or southern part of Portugal, at approximately the same latitude where the first SBV case in Spain occurred in 2012. Previous studies on the spatial distribution of *Culicoides* spp. in Portugal have shown that these vectors are mainly conformed to the central/southern regions of the country.

Epidemiology

P03-003-073

Bovine papillomavirus and its heterogeneous distribution in different host species in Italy

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Objectives: Papillomaviruses (PVs) are a wide variety of epitheliotropic viruses responsible for benign lesions' development that can occasionally progress to cancer. Even though PVs are considered highly species and site specific, Delta-genus Bovine papillomaviruses (BPVs) are known to cause cross species infection giving rise to sarcoids in horse and cat. At the light of the hypothesis that cross-species transmission has played an important role in PVs evolution, we evaluated the distribution of BPVs in their natural host and in diversely related species in Italy to deepen knowledge about tropism and transmission.

Materials and Methods: Cutaneous proliferative lesions clinically resembling papillomas on different anatomical sites of beef and dairy cattle, as well as healthy skin and mucosa from red deer, roe deer, chamois and mouflon, were collected from vet practitioners in Italy. Sampling was conducted in northern, central and southern regions. After DNA extraction, analysis for the identification of different BPVs was conducted by PCR with degenerated primers able to amplify specific genera and types.

In order to amplify also genomic DNA of unknown PVs, RCA (Rolling Circle Amplification) isothermal amplification and enzyme restriction analyses were also performed. Amplified products were subsequently sequenced and characterised.

Results: Papillomatous lesions demonstrated to harbor the genome of at least one PV. Interestingly, the diffusion was not homogeneous, as different genera were variously distributed on the territory, and many samples presented coinfections between types and genera. In wild ruminants, despite sampling of healthy tissues, an irrelevant number of samples harbored host specific papillomaviruses, while we extensively detected BPVs' DNA. Surprisingly, not only Delta BPVs, already known to cause cross-species infections, but also other genera were amplified from skin and mucosa.

Conclusions: Findings of BPVs presence in cattle lesions demonstrate a disomogeneous distribution of different genera ad types on Italian territory. In addition our results point out that the ability to cross-infect other species that was previously assumed only for Delta PVs, is potentially conceivable between other BPV genera. In conclusion our data contribute to a better understanding of papillomavirus epidemiology considering the possibility of indirect transmission between wild and domestic specie, and give further information about the role of sub clinically infected animal species.

Epidemiology

P03-003-074

Using Network Metrics to Predict Disease Spread from animal movements of the Cattle Tracing Scheme

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Objectives: Our work had two **Objectives:** Firstly, to investigate cattle movement network to identify individuals and structures where control will have greatest effect on maintenance and transmission of disease; Secondly, to use the Cattle Tracing Scheme (CTS) to investigate the relative contribution of local movements and movements across regional boundaries to the risk of spreading diseases.

Materials and Methods: We used the CTS data to construct monthly contact networks of animal movements to and from individual holdings. The holdings were described by their business type (e.g. Market or Agricultural Holding). A variety of metrics (including betweenness, degree and clustering coefficient) were calculated to help describe the networks and to predict the influence each type of holding would have on disease spread. A high value of betweenness (the number of times a holding lies on the shortest path between two other holdings) has been used by others to predict which holdings are important in disease spread.

To establish the relative contribution of local versus regional movements to disease spread we used the county boundaries to partition the networks into within (intra) county and between (inter) county.

Results: Our main findings were that the average values for betweenness for the holdings described as Calf Collection Centre, Market and Showground are much higher than the other holdings. We also found strong (and unsurprising) evidence of seasonality over all holdings. The seasonality doesn't hold for all business types (e.g. Calf Collection Centres) but does hold for some (e.g. Markets).

Partitioning the network into intra county movements and inter county movements provides further information. The pattern of movements is consistent between the two partitions and the betweenness values from networks based on inter county movements are much larger than those based on intra county movements.

Conclusions: If betweenness is an indication of disease spread (as literature strongly suggests) then stopping inter county movements will have a bigger effect than stopping intra country movements. Likewise Calf Collection Centres, Markets and Showgrounds will have a larger influence on disease spread than the other types of holding.

Epidemiology

P03-003-075

Risk Factors For Calf Mortality During The First 24 Hours Of Life On Dairy Farms In Hokkaido, Japan, 2005 To 2009

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Objectives: This was a retrospective cohort study using data from the

insurance scheme provided by the Japanese Mutual Aid Association (NOSAI). The population of interest comprised all cattle born on NOSAI-client farms in the Japanese prefecture of Hokkaido, Japan for the period 1 January 2005 to 31 December 2009 (inclusive). Our aim was to identify risk factors for mortality in calves that were either stillbirth or born alive but died during the first 24 hours of life.

Materials and Methods: Data were retrieved in digital format from the Hokkaido NOSAI office for all calves born throughout the study period. Details for each calf included the national identifier of the farm on which it was born, its date of birth, the national animal identifier of the calf's dam, the parity of the dam at the time of delivery, the number of calves delivered by the dam, and calf breed. A mixed-effects logistic regression model was developed to identify explanatory variables associated with first 24 hour mortality risk, with calves clustered within dams and dams clustered within herds. The final data set comprised details of 1,281,737 births on a total of 5,172 dairy herds from 19 NOSAI branches located throughout the prefecture of Hokkaido.

Results: Over the five year study period there were 7.39 (95% CI 7.35 to 7.44) deaths for every 100 deliveries. Factors that were positively associated with an increase in first 24 hour mortality risk included being born in the colder months of the year (November to March), calves of Wagyu breed, having a multipara dam, multiple (as opposed to single) birth deliveries, and increases in herd size. After adjusting for the fixed effects included in our multilevel model 88% of the unexplained variation in first 24 hour mortality risk was at the individual calf level.

Conclusions: We propose that the data recording requirements of the NOSAI scheme are extended to include details of calving events (e.g. the presence or absence of dystocia) and details of the way in which calves are managed post delivery. This would allow more subtle risk factors for calf mortality to be identified which, in turn, will lead to refinement of recommendations of the way calves should be managed during the first 24 hours post delivery.

Epidemiology

P03-003-076

Associations between Clostridium botulinum and unspecific chronic health problems in dairy herds – a case control study

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Objectives: Since a couple of years, chronic herd health problems in dairy cows accompanied by many unspecific clinical symptoms eventually leading to long infirmity or death have been observed in Germany. Botulinum neurotoxin (BoNT) was found in the gastrointestinal tract and other organs of diseased animals. Therefore, a toxicoinfection with Clostridium (C.) botulinum was postulated and the disease was named chronic or visceral botulism. In order to study possible associations between chronic disease problems in dairy herds and C. botulinum or its BoNT, a case control study was performed. C. botulinum or the BoNT were considered the main risk factor.

Materials and Methods: Case farms had a history of chronic disease problems in the past and for verification, had to fulfill 3 out of 5 inclusion criteria: decreased herd milk yield (> 15%), increased culling rate (> 35%), increased death rate (> 5%), increased incidence of downer cows (> 10%), overall increased disease rate. Control farms (n=47) did not fulfill any of those criteria. Case 1 farms (C1: n=45) did not vaccinate against Clostridia, whereas Case 2 farms (C2: n=47) vaccinated against Clostridia, but not against *C. botulinum*. On each farm, 5 chronically ill cows and 5 unsuspecting cows underwent clinical examination and sampling (feces, ruminal fluid) (total n=1389). In addition, silage and water samples (n=406 and n=139, respectively) were collected. Clostridia diagnostic included anaerobic culture of fecal, ruminal fluid, silage and water samples with species differentiation using 16S rRNA sequence analysis, identification of BoNT toxin genes using singleplex real time PCR (all samples) and conventional singleplex PCR (fecal samples only) and identification of BoNT in fecal samples using mouse bioassay. A farm was considered positive when at least one animal sample tested positive in at least one of the assays. A cow was considered positive when at least one sample (feces, ruminal fluid) tested positive in at least one of the assays. Statistical analysis included Chi-square test or logistic regression.

Results: Out of a total of 7.934 investigated and suitable Clostridia isolates, only one was identified as *C. botulinum* type B. This isolate came from a control animal of a C2 farm. None of the fecal samples tested positive for BoNT. With respect to BoNT gene, 23.7% of the farms (C1: 22.2%; C2: 27.7%; control: 21.3%) were considered positive. The odds ratio for the occurrence of *C. botulinum* on herd level was 1.09 (CI: 0.48-2.49; P=0.74). On cow level, 109 cows (7.9%) were positive (C1 sick 6.2% vs healthy 5.8%; C2 sick 11.1% vs healthy 9.4%; control sick 6.4% vs healthy 8.1%). The frequency of positive results did not differ between animal groups within farm category or between farm category within animal groups (P>0.05). Out of 406 silage samples, only 11 (2.7%) from 9 farms had BoNT gene detection (C1: 9.8%; C2: 4.3%; control: 6.4%; P>0.05).

Conclusions: The fact that no BoNT was detected and toxin genes on herd and cow level were present in control and case farms to the same extent, strongly suggests that there was no direct and clear association between the occurrence of *C. botulinum* and chronic unspecific diseases in dairy herds. Therefore, it is not likely that *C. botulinum* is the main exposition factor for the problems on the affected farms. In addition, silages which are often discussed as a source of *C. botulinum* apparently played an inferior role in this respect. In conclusion, the results of this study did not give evidence for the existence of chronic/visceral botulism.

Epidemiology

P03-003-077

Differential diagnosis of the bovine neurologic disease in Paraná State, Brazil: between 2009 and 2015

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Objectives: The objective of this research was to investigate the occurrence and the causes of neurologic diseases in cattle in the State of Paraná, Brazil, from February 2009 to November 2015, establishing a differential diagnosis for rabies.

Materials and Methods: This research was conducted in the Veterinary Hospital of Londrina State University (HV-UEL) and in the farms where the diseases occurred. All the information about the evolution of the diseases in the individual and in other sick animals from the herd were recorded, as well as all the risk factors involved, and other relevant management information. All the procedures such as physical examination, neurologic examination, material collecting, preservation and processing were followed by a standard protocol. The following samples were collected from live cattle: blood, with and without anticoagulant and cerebrospinal fluid.

The necropsies were complete and tissues samples of all organs, including the central nervous system were collected including ganglion of the trigeminal nerve, carotid network mirabile and pituitary, and if necessary, the spinal cord.

The brain was collected and processed for histopathological examination, for polymerase chain reaction for detection of BoHV-1 and BoHV-5 genomes. Likewise, in each case attended, brain samples were sent to Diagnostic Center Marcus Enrietti (CDME) for the diagnosis of rabies, and if the animals were over 24 months of age, samples of brainstem preserved in formaldehyde were sent for diagnosis of BSE.

Results: Two hundred and nine cases were observed, either isolated cases or outbreaks, belonging to 123 different herds located in 77 counties. Approximately 90% of cases occurred in beef cattle. Of all cases investigated, only 7% remained inconclusive. Inflammatory and toxic diseases were the most frequent and accounted for 44% and 32% of cases, respectively.

Inflammatory causes such as meningoencephalitis caused by BoHV-5 (36%) and rabies (32%) were the most diagnosed diseases. Other diseases included thrombotic meningoencephalitis by *Histophilus somnus* (n=8), cerebral babesiosis (n=4), Listeriosis (n=2), BoHV-1 encephalitis (n=2) and Malignant Catarrhal Fever (n=2). Rabies occurred more often in the form of outbreak and BoHV-5 encephalitis most commonly in the form of isolated cases. Rabies affected cattle from various ages, while BoHV-5 encephalitis affected young cattle, less than 20 months. The most common degenerative cause of neurology disease was polioencephalomalacia, with 9 cases.

Toxic diseases consisted of 68 cases, the most important causes being the poisonous plants *Senecio brasiliensis* (n=8), *Crotalaria* spp. (n=7), *Senna obtusifolia* (n=6), *Tabernaemontana catharinensis* (n=5), *Senna occidentalis* (n=4). The tremorgenic syndrome by *Cynodon nlemfuensis* and *Cynodon dactylon* occurred in 8 cases, and nitrate/nitrite poisoning, in 5 animals. Carbamate, organophosphate, urea and mycotoxins poisonings were observed only one case each. Six cases of botulism, two cases of tetanus and two cases of enterotoxemia completed this list.

Conclusions: The conclusion of this research is that viral encephalitis, like rabies and BoHV-5 are the most important causes of bovine mortality in Paraná State. Followed by a large list of plant poisoning. Bacterial diseases have little epidemiological importance, presented as isolated cases. Listeriosis is a great cause of mortality in the northern hemisphere, but in this study only two isolated cases were reported.

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Epidemiology

P03-003-078

Bovine Respiratory Syncytial Virus Seroprevalence Study In Bovine And Non-Bovine Species Of Agricultural Importance In Argentina

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Objectives: Bovine respiratory syncytial virus (bRSV) is the main cause of respiratory disease in young calves. The virus has been reported in many countries either by viral isolation or serologic studies. In Argentina, little is known about bRSV infection in cattle and the role of non-bovine species in the epidemiology. With the aim of evaluating bRSV circulation in both dairy and beef cattle and in non-bovine species (camelids, buffaloes, sheep and goats), a seroprevalence study was performed.

Materials and Methods: Cattle herds from Buenos Aires, Córdoba and Santa Fe provinces, in Argentina, were chosen for this study. In the case of beef and dairy herd, animals were random selected stratified by two age classes (6-18 months old and above 18 months). The first group involved 250 animals from 25 herds and the second group comprised 125 animals from the same herds. Antibodies against bRSV were also evaluated in cattle from 12 feedlots. Serum samples were taken from 230 animals older than 6 months in order to avoid colostral antibodies interference. To study the role of non-bovine species in epidemiology, sera were obtained from 10 llamas from Jujuy; 173 buffaloes from Corrientes, 36 sheep from 3 farms located in Jujuy; 60 goats from 6 farms located in a peri-valley area in the north of Rio Negro and 91 goats from 3 farms in Salta, all provinces in different regions of Argentina. None of the animals had been vaccinated against bRSV before sampling.

Results: Serum antibodies against bRSV were detected by virus neutralization test. Results showed that all herds had at least one positive animal for bRSV. The individual apparent seroprevalences were: 74.6% (6-18 months) and 97.9% (>18 months) in dairy cattle, 78.2% (6-18 months) and 94.9% (>18 months) in beef cattle and 78.6% in feedlot cattle. Old animals had the highest odds of being bRSV seropositive. Regarding non-bovine species, antibodies against bRSV were found in goats, from Rio Negro and Salta with seroprevalences of 86.9% and 95.5%, respectively. All sheep evaluated showed antibodies against the virus. Concerning buffalo samples 43% were positive for bRSV while 60% of the llamas showed low antibody titers against the virus.

Conclusions: Results presented here show that the virus is widely distributed not only among cattle but also in non-bovine species which might be involved in the epidemiology and transmission of bRSV. The presence of antibodies in non-vaccinated animals demonstrate that bRSV is circulating in our country so that control measures should be taken into account in order to prevent respiratory disease caused by this virus.

Epidemiology

P03-003-079

Applying Haddon's matrix to bovine injury prevention: An example using white line disease

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Objectives: Haddon's matrix is a model used to conceptualize injury occurrence. This model defines injury as energy transfer, by the agent to the host, in quantities or rates exceeding the tolerance of the host's tissue. While this approach has been used in human injury research for over 30 years to identify risk factors and develop preventive interventions, we have not seen it applied to animal injury. Lameness, an etiologically complex condition, is a source of both economic losses and welfare concerns to the cattle industry. We introduce Haddon's matrix as an approach to viewing traumatic animal injury using bovine white line disease as an example.

Materials and Methods: First, we identified three temporally distinct and successive phases of the injury process, each corresponding to a row of the matrix: The pre-event phase (the period prior to the transfer of mechanical energy to the white line), the event phase (the period during which mechanical energy is being transferred to the white line) and the post-event phase (the period subsequent to the transfer of mechanical energy to the white line). Second, we defined four interacting factors that contribute to an injury event, corresponding to the columns of the matrix: The host that sustains the injury (e.g. the cow); the agent, the object that transfers the energy to the host (e.g. a foreign body such as a stone); the overall physical environment in which the injury occurs (e.g. the farm, including the design of housing, floor surfaces and roadways, presence of pasture and herd size); and the socio-cultural environment or context: herd management (e.g. lameness control strategies, methods of herding etc.), cultural practices (e.g. awareness and attitudes to lameness) and animal welfare regulations. Finally, we used each cell of the 3 x 4 matrix as a focus for brainstorming and, guided in part by current literature, identified factors which could potentially a) prevent, b) limit the quantity of and c) reduce the consequences of energy transfer by various agents to the white line.

Results: The matrix enabled examination of factors as they pertained to specific phases of the injury process and thus facilitated evaluation of the feasibility of different phase-specific injury countermeasures. Thus it helped to compartmentalize a seemingly complex problem into manageable segments. Specifically, we found most potentially successful countermeasures applicable to either the prevention of energy transfer (pre-event phase) or the limiting of its consequences (post-event phase). Few event phase countermeasures were identifiable and deemed potentially capable of sufficiently limiting the quantity of energy being transferred to adequately protect the white line from injury. Additionally, each host, agent and physical environment countermeasure could be conceived as temporally consequent to a particular socio-cultural environmental (management) countermeasure. Finally, each host countermeasure, for example improving horn quality, had an analogue at the management level.

Conclusions: Haddon's matrix facilitates clear thinking while preserving a multidimensional approach to preventing white line disease. Given the impossibility of protecting the white line tissue once energy above a certain threshold is released, efforts should be expended primarily to prevent the release of energy in those quantities, and secondarily to reduce its consequences. Management-related countermeasures, being earliest in the causal pathway, are likely to be most influential in preventing white line disease and should take priority as preventive strategies. This is consistent with recent recommendations made by other authors.

Epidemiology

P03-003-080

The use of geographical information system for the surveillance of Bluetongue in the district of ASL Sassari (Sardinia)

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Objectives: Surveillance and prophylactic measures both direct and indirect are the means against Bluetongue. Through the surveillance system, data is acquired to provide information to show the presence or absence of viral circulation and to assess the risk related to the movement of sensitive species. The purpose of this paper is to present our experience on the use of GIS technology to provide information on the limitation of viral shedding and consequent ability to undertaken an early assessment of risk to health in the district of the ASL of Sassari.

Materials and Methods: Data was examined during the surveillance period from 2012 to 2015. Farms were selected for inclusion by the investigators if they partook in provisions of the National Manual for the Bluetongue Surveillance (April 2007). Using the GIS ArcView 9.1, the district of ASL Sassari was divided into 14 cells, 20 km², representing the geographical unit of reference for the surveillance activities. Within these areas the selected farms and the number of animals (including sheep, cow and goat) were monitored monthly for the presence of antibodies against the virus BT on serum samples.

This allowed us to acquire a monthly incidence of seroconversion of 2% with reliability of 95%.

The animals used as sentinel came from areas which had not been vaccinated and where there was no viral circulation. Blood samples were collected from the farms by the investigators, and subsequently analysed using the ELISA method of serum neutralisation to test for the BT virus. In 2012, 72 blood samples were collected every month from 10 bovine farms, 581 blood samples from 31 sheep farms and 32 blood samples from 3 goat farms. In 2013, 65 blood samples were collected every month from 8 bovine farms, 514 blood samples from 32 sheep farms and 23 blood samples from four goat farms. In 2014, 56 blood samples were collected every month from seven bovine farms, 477 blood samples from 27 sheep farms, 46 blood samples from 2 goat farms. In 2015, 36 blood samples were collected every month from 4 bovine farms, 382 blood samples from 19 sheep farms and 365 blood samples 16 goat farms.

Results: The results show that in 2012 there were no positive cases for any serotype. However, the situation changed in the following years. In 2013 for serotype 1, 24.31% of sheep, 89.23% of the bovines and 39.13% of goats tested positive.

In 2014 a small percentage (0.83%) of sentinel sheep, 32.14% of sentinel bovines and 2.17% of sentinel goats tested positive to serotype 1. In 2015, 2.77% of sentinel bovines and 3.56% of sentinel goats tested positive for the same serotype. For each animal seropositive with Bluetongue a cartographic buffer region of 4km² was enforced around the farm of origin. Within these areas of restriction animals identified as being species susceptible to bluetongue were subject to blocks in animal movements to prevent a possible viral spread. The process of geo-referencing has also allowed us to extrapolate on Microsoft Excel spreadsheets containing all owners of these farms and the corresponding phone numbers to send, via the Gateway software, a text message alert to update the receivers on the epidemiological situation of breeding and measures of restriction in place.

Conclusions: Through the use of GIS it has been possible to improve the surveillance program of Bluetongue, to update and view in real time, the epidemiological situation on cartographic basis for circulating serotypes.

By blocking the movement of animals from farms identified as being a source of Bluetongue, the system has made a large contribution to the elimination in 2010 of bluetongue virus serotype 4 and in 2011 the elimination of bluetongue virus serotype 2 from the territory of the ASL of Sassari.

Epidemiology

P03-003-081

Determination Of The Analytic Sensitivity Of An Elisa Indirect Test In Pool Serum For The Detection Of Bovine Brucellosis

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Objectives: Bovine brucellosis (Brb) is a worldwide distributed disease with a public health importance and economic impact. In Uruguay the prevalence of the disease goes around 0.8% among farms, in this stage of advanced control, there are several diagnostic tests been implemented in surveillance such as, Rosa Bengala test (RB) implemented for individual animals and ELISA in milk pools. According to different authors the ELISA test has a high sensibility for this disease, therefore the possibility of making serum pools. The objective of this study was to determine the analytic sensitivity of an indirect ELISA test in serum pools.

Materials and Methods: 62 positive serum samples to the RB test and confirmed by Rivanol or Fluorescent Polarization test were used in this study. These samples were diluted with negative sera from animals that did not belong to farms with reactors for the disease, this sera were also negative to RB test. The dilutions were carried out in a 2 base up to 1/1024. The ELISA test was applied to original samples and the dilutions to determine the analytic sensibility of the pool samples. The results were analyzed by a Probit model in a STATA 13.0 software.

Results: The mean of the analytic sensibility for the dilutions are: 1/4 (98.2%), 1/8 (96.8%), 1/16 (94.4%) and 1/32 (90.9%), if the serum is diluted to greater proportions the analytic sensibility decrease. The use of an ELISA test in pool serum can be implemented as a strategy for the declaration of farms free of Brb, since the probability of detecting a positive pool is more likely to be low in the country disease situation. Considering the costs of both tests (RB and ELISA) the cost of the RB test goes around 1USD per sample, and the cost of the ELISA is 2USD, beside of this in the situation that the pools are made of 16 samples the national cost of the diagnostic campaign decrease in 8 times. In total in Uruguay with the sanitary obligations for the dairy cows, the beef farms that are under control or side to control farms and the sampling in slaughterhouse the number of animals analyzed per year goes around 2 million.

Conclusions: With a campaign that annually analyzes more than 2 million individual samples, in a country perspective of 0.8% prevalence among farms, the probability of obtaining a positive pool is low and the need to analyze a pool as an individual sample is unlikely. In this national campaign, the cost of the test been implemented are paid by

the farmer, with the implementation of pool samples this will result in lower production cost to agricultural producers and less number of staff needed in the laboratories.

Epidemiology

P03-003-082

Prevalence study on diseases observed on cattle for slaughter and on the causes of exclusion of the meat for human consumption

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Objectives: The purpose is to collect clinical and anatomo-pathological evidences in the slaughterhouse and to analyse the capacity of the inspection for reveal the diseases and the other conditions recognized as a ground for excluding beef from human consumption. This study investigates the role of the slaughterhouse as an epidemiological observatory.

Materials and Methods: The study has been conducted for 45 months on cattle slaughtered in the establishment IT034MCE current in the Province of Teramo (Italy), from 01.04.2011 to 31.12.2014. The observed cattle population is composed of 14512 bovines. The animals only in a small percentage come from the territory next to the slaughterhouse (25-30%), the rest is of national or Europe Union origin. The data of the results of inspections and decisions concerning live animals and meat have been registered in the database provided for by EC Reg. 854/2004. There have been considered the clinically cases of disease confirmed during ante and post mortem inspection, and the causes of exclusion of meat from human consumption. In addition to the absolute number of cases, it has been calculated the prevalence in comparison with the animals inspected. There have been considered "cases" all the ones confirmed by the official veterinarian. The cases have been classified according to the categories of diseases and the causes of exclusion of meat from human consumption provided for by the art. 5, Annex I, Section I, Chapters I, III and V of EC Reg. 854/2004.

Results: From 14512 cattle slaughtered 752/5,18% has been suffering from disease. Among the sick ones 19/2,53% has had generalized illness; 13/1,73% has been emaciated; 143/19,01% has been suffering from hydatid disease (zoonosis); 161/21,41% from other non-zoonotic parasitosis; 307/40,83% has been suffering from aspecific localized inflammations and 103/13,70% from degenerative diseases. In 6/0,80% cases other factors have required that the meat had been declared unfit for human consumption. There have been 753 animals of which meat has been excluded. The exclusion has been total in 37/4,92%. 1/0,13% bovine has died before slaughter, 19/2,52%, has been suffering from general disease, 13/1,74% has been emaciated, in 1/0,13% the bleeding has been insufficient and in 3/0,40% cases the meat has presented organoleptic anomalies. It has been partial in 716/95,08% cases: in 404/53,65% for physiopathological alteration of single organs or systems; 307/40,77% for parasitosis of single organs. In 5/0,66% cases the exclusion of the meat has been due to technological errors (foulness). Among the noticed diseases prevail the located ones (parasitic or related to inflammatory aspecific processes) for which it is sufficient the exclusion from the consumption of the only affected tissues or organs. The control in the slaughterhouses has been efficacious as regards the prevention of zoonosis as hydatid disease and the identification of animals suffering from systemic diseases (septicemia, pyaemia, viremia, toxemia) that can make dangerous the meat of the whole carcass for the human consumption.

Conclusions: The slaughterhouse is still an epidemiological observation post essential it reflects the epidemiological situation of populations. The routine inspection along with informations on the food chain effectively pursues the objectives of consumers protection. However only in the case of animals suspicious of disease that may negatively affect human or animal health will follow a thorough examination to obtain an etiologic diagnosis. In the other cases the sensitivity of the inspection method and therefore the role of epidemiological observatory as regards non-zoonotic diseases or for which there aren't provided for measures of health are reduced.

Epidemiology

P03-003-083

Liver fluke (*Fasciola hepatica*) infection in cattle in Northern Ireland: a large-scale epidemiological investigation utilising meat inspection data

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Objectives: Liver fluke (*Fasciola hepatica*) is a widespread and common parasite of ruminants in Northern Ireland. Liver fluke infection status (for non-clinical cases) at the animal level is captured during meat inspection of all animals processed for human consumption within Northern Ireland. These large datasets have not been analysed to assess their utility in uncovering trends in fluke infection at animal and herd levels in Northern Ireland.

Materials and Methods: We utilised a dataset of 1.2 million animal records from 18000 herds across three years (2011-2013) to assess animal and herd level apparent prevalence and risk factors associated with fluke infection. Data from routine meat inspection surveillance were gathered from the Animal and Public Health Information System (APHIS) for Northern Ireland. Appropriate data cleaning was undertaken before exploratory descriptive analysis. Animal level prevalence was measured as the proportion of animals exhibiting evidence of fluke infection at slaughter; herd-level infection prevalence was measured by categorising herds into infected or not (binary), if at least one animal exhibited at least one infected animal per unit time. "Within herd" infection prevalence was measured using the proportion of animals within a herd that showed evidence of fluke infection (ranging from 0-1), with the inclusion criteria of a minimum of 13 animals sampled per herd per year. "Within herd" infection prevalence at the herd level was investigated using logistic regression, Generalised Linear models (GLM) and Fractional Response Regression (FRR), with spatial patterns presented using Geographic Information Systems (GIS).

Results: At the animal level, the proportion of animals slaughtered that exhibited evidence of infection varied between 20-25% amongst years. Across herds, the proportion of herds with at least one infected animal, varied between 60-65%. There was however a significant sampling effect at the herd-level; all herds where at least 105 animals slaughtered over the study period exhibited evidence of fluke exposure (100%).

There was significant variation in terms of within-herd infection prevalence risk as elucidated by GLMs and FRRs. Risk factors included herd type, long-term climate variation, geographic location (region) and the abattoir where animals were processed.

Conclusions: Liver fluke prevalence was high at the herd level. However, there was a lower prevalence at the animal level, which may indicate variation in exposure within herds. Within herd prevalence varied significantly in time and space, and by abattoir, herd-type and some climate variables. These data are a useful source of information on a widespread and endemic disease, despite known limitations in terms of performance (low test sensitivity). As well as informing on the distribution and severity of liver fluke infection within herds in Northern Ireland, these analyses will be used to investigate the effect of co-infection with bovine tuberculosis.

Epidemiology

P03-003-085

Prevalence of *Coxiella burnetii* (Q fever) in Polish cattle population

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Objectives: Q fever is an infectious disease of animals and humans. The biggest worldwide outbreak of Q fever took place between 2007 and 2010 in the Netherlands and initially it was considered as the cause of increasing prevalence in other European countries. Recent phylogenetic analyses show that strains isolated from cows from other European cattle belong to different cluster than the Dutch one. Increasing number of Q fever cases is probably due to the lack of surveillance and eradication programs in most of Member States. There for the aim of this study was to assess the prevalence of *Coxiella burnetii* in cattle herds in different regions of Poland.

Materials and Methods: The study was performed at the turn of 2014-2015 on 1,150 serum samples collected from cattle in different provinces and counties. The samples were taken from 443 cattle herds. The sera were tested by complement fixation test (CFT). CFT assay was done using reagents supplied by Siemens (Germany) according with manufacturer's instruction. The mixed antigen of *C. burnetii* (phase I and phase II) was used. Moreover, bulk tank milk (BTM) were taken from 25 seropositive cattle herds and tested by qPCR. Additionally, 30 commercial milk and dairy product were tested by PCR. DNA isolation from milk and dairy products will be performed with use of commercially available kit QIAamp DNA Mini Kit, according to the manufacturer's instructions. For the qPCR test, a commercial kit targeting the repetitive transposon-like region (IS1111) of *C. burnetii* (AdiavetÖCOX REALTIME) was used. Samples with cycle threshold (Ct) values below 37 were positives.

Results: The data indicated that *C. burnetii* serologically positive cattle was detected in all provinces under this study. The percentage of seropositive herds range from 15.79% in Warmińsko-Mazurskie province to 84.62% in the Mazowieckie province with an average of 40.41%. Surprisingly, the seroprevalence below the national average was in Podkarpackie (29.91%) which is included in the endemic area of Q fever. Presence of repetitive transposon-like region (IS1111) was confirmed in 8/25 (32%) BTM. The Ct value for positives BTM was ranging from 28.8 to 36.8. Moreover, in ten commercial milk and dairy product positives results of qPCR were noted.

Conclusions: The data presented in this study shows that *C. burnetii* is widespread in Poland, and the surveillance has to be continued. Especially as Q fever is zoonotic disease and there is a high risk transmission to humans. Particularly that transmission to humans by alimentary route cannot be ruled out.

Epidemiology

P03-003-086

Evaluation Of The Implementation Of The Program Of Control Of Bovine Brucellosis In The Manabi Province, Ecuador

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Objectives: In Ecuador there is a national program to combat brucellosis is voluntary compliance and therefore the application level is not known, which is why we propose aim of this study to know the level of compliance with the measures ordered by the in the Manabi province.

Materials and Methods: A cross-sectional epidemiological study was conducted in the period November 2014 to March 2015 in cattle herds of Chone, El Carmen, Sucre, Jama, Bolivar, Tosagua and Junin cantons of Manabi province. For determining compliance with the aspects covered by the national program for control of bovine brucellosis (AGROCALIDAD, 2009) an epidemiological survey in 20 affected herds and 20 unaffected herds randomly selected in the territory was applied.

The factors taken into account in the assessment of compliance were: proper use of vaccination, have veterinary assistance, investigation of herd annually, development of surveillance activities, removal or compulsory segregation of positive reactors, sanitary control of entry and exit of animals, biosecurity and herd development of health education activities.

The data were recorded in a spreadsheet of Microsoft Excel. The percentage of compliance with each of the aspects referred to in brucellosis control program was calculated and the level of compliance among affected and unaffected herds was compared using the chi-square test and statistical package Vassarstats.net.

Results: A low compliance of the measures proposed in the control program was determined, only the veterinary assistance measure obtained more than 50% of compliance, in the rest of the aspects it was demonstrated a very low compliance level (table 1).

Table 1. Compliance level of the different aspects covered in the National Programme for Control of Bovine Brucellosis in the studied territory of Ecuador.

Measures	Compliance (%)
Vaccination	15.00
Veterinary assistance	67.50
Periodical test of the animals	0.00
Serological Test at the animals entrance into the herd	27.50
Epidemiological Surveillance	2.50
Culling of infected animals	5.00
Biosecurity	5.00
Health Education	7.50

It was found that herds free of the disease showed a better performance in their compliance with the measures, veterinary care and serological testing of animals before they enter the herd. It was also found that 100% of vaccinated herds were not infected, demonstrating the importance of this measure.

Conclusions: There is a low level of compliance in control measures of bovine brucellosis in the areas studied in the Manabi province of Ecuador. The importance of vaccination on disease control was demonstrated in this article.

Epidemiology

P03-003-087

Seroprevalence of *Mycoplasma bovis* in affected cattle population in Poland

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Objectives: *Mycoplasma* spp. belongs to Mollicutes class. Among the bacteria they have the smallest genome size. *M. bovis* is thought to be the main pathogen of calf pneumonia. *M. bovis*-associated pneumonia may occur in cattle in different age. *M. bovis* also can cause pathological changes in the mammary gland, joints, conjunctival sac, ears, infertility and abortions. It causes significant economic losses in cattle industry. The aim of this study was to assess the seroprevalence of *Mycoplasma bovis* in cattle herds that indicate the symptoms of mycoplasma infection derived from different regions of Poland.

Materials and Methods: The study was performed during 2014 – 2015. In total 713 serum samples from 73 cattle herds were examined. There were examined from 6 to 75 animals from each herd (on average 10 animals from a herd) affected with pneumonia, arthritis or mastitis. The animals derived from different regions of Poland. The presence of specific anti-*M. bovis* antibodies in the tested serum samples was determined using the commercial indirect ELISA. The sera were tested by ELISA (BioX Diagnostics) according to manufacturer's instruction.

Results: The presence of anti-*M. bovis* antibodies was detected in 30.14% (22/73) herds. The percent of the positive herds ranged from 0 to 45.5% regarding to the region. The highest result was recorded in Northwestern Poland – 45.5% (5 herds from 11), lower results were recorded respectively in: Central Poland – 42.9% (6/14), Sothwestern Poland – 40% (2/5), Eastern Poland – 36.4% (8/22) and North Poland – 7.14% (1/14). In South Poland the presence of anti-*M. bovis* antibodies was not detected in any of the 7 herds.

Conclusions: The data presented in this study shows that anti-*M. bovis* antibodies are present in Polish cattle population at a significant level, which can vary regarding to the region. To define the presence of active infection of *M. bovis* in the herd, the examinations should be expanded to the bacteria detection with the use of culture and molecular methods.

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Epidemiology

P03-003-088

The upper respiratory tract microbiome and its potential role in bovine respiratory disease and otitis media

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Objectives: The objective of this study was to use high-throughput sequencing of the 16S rRNA gene to characterize the microbiome of the

URT of Holstein dairy calves from day 3 to day 35 of life and to describe its associated bacterial diversity. In addition, we aimed to compare the microbial community of the URT of healthy and unhealthy subjects at each time point of our data collection.

Materials and Methods: A cohort study was conducted including 174 calves categorized prospectively as healthy, pneumonia only, otitis only, or pneumonia-otitis combined. Deep pharyngeal swab samples were collected on days 3, 14, 28, and 35 of life. Isolation of DNA from swabs of the URT was performed. The 16S rRNA gene was amplified by PCR from individual metagenomic DNA samples from the URT using barcoded primers. For amplification of the V4 hypervariable region of the bacterial/archaeal 16S rRNA gene, primers 515F and 806R were used according to previously described methods and optimized for the Illumina MiSeq platform. Aliquots of URT amplicon samples were standardized to the same concentration and pooled into 5 different runs according to individual barcode primers for the 16S rRNA gene. Final equimolar libraries were sequenced using the MiSeq reagent kit v2 (300 cycles) on the MiSeq platform. The generated 16S rRNA gene sequences were processed through the open source software pipeline Quantitative Insights Into Microbial Ecology (QIIME) version 1.7.0-dev. Sequences were binned into Operational Taxonomic Units (OTUs) based on 97% identity using UCLUST against the Greengenes reference database. We generated a species-level OTU table using the MiSeq Reporter Metagenomics Workflow. In order to determine the bacterial load of the URT samples and its difference between each health status examined we performed a qPCR assay.

Results: Our results showed that the URT microbiome is dominated by the phyla Proteobacteria, Tenericutes, Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Spirochaetes, and Cyanobacteria. Thirty genera were shared across the four age groups and health statuses. At days 14 and 28, the relative abundance of the genus *Mannheimia* increased in calves with pneumonia and pneumonia-otitis combined compared to healthy animals. At day 28 alone, the relative abundance of *Mycoplasma* increased in calves with pneumonia and pneumonia-otitis combined compared to healthy calves. Interestingly, *Moraxella*, a bacterium not previously associated with pneumonia and otitis in calves, showed increased relative abundance at day 14 in animals diagnosed with pneumonia, otitis and pneumonia-otitis combined. *Pasteurella*, a bacterium previously reported to be associated with bovine respiratory disease and otitis, did not exhibit a significant difference in abundance between healthy calves and calves diagnosed with pneumonia, otitis or pneumonia-otitis combined.

Conclusions: This study demonstrated that the URT of calves across the four ages examined harbored a similar microbial structure regardless of health status (suggesting that a commensal microbiota is dominant), an increase in the likelihood of developing respiratory diseases leads to variation in some specific microbe abundances. Both age and pathogenic agents appear to be important in determining the health of the calf respiratory tract. For the first time, *Moraxella* was associated with pneumonia and otitis in pre-weaning dairy calves, which call for further investigation into its potential role on the health of the respiratory tract and middle ear.

Epidemiology

P03-003-089

The prevalence and risk factors for antibodies to *Coxiella burnetii* (Q fever) in bulk tank milk samples from Irish dairy cattle herds

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Objectives: *Coxiella burnetii* is the causative agent of Q fever, a zoonotic disease of increasing public health importance which also causes abortion and fertility problems in cattle. The objective of this work was to estimate the prevalence of antibodies to *C. burnetii* in samples of bulk tank milk collected from Irish dairy farms, to determine whether positive herds clustered spatially, and to investigate the strength of association between certain putative risk factors and positive status.

Materials and Methods: Bulk tank milk (BTM) samples from Irish dairy farms were collected by dairy processors during the summer of 2015 in the context of a collaborative initiative between the dairy industry and the Irish Veterinary Laboratory Service of the Department of Agriculture, Food and the Marine (DAFM). 1,489 BTM samples were randomly selected from the total of 14,464. The survey was designed based on prior estimates of true prevalence of 0.35 (based on work conducted in 2010), test sensitivity of 0.9, test specificity of 0.9, population size (number of dairy herds participating) of 18,000, confidence of 0.95 and precision of 0.03, using epitools software (www.epitools.eu). BTM samples were tested using a commercial ELISA kit according to the manufacturers' instructions. Herd level data extracted from the DAFM animal identification and movement (AIM) database is used to evaluate herd-level risk factors for positivity, with spatial analysis conducted using ArcGIS.

Results: 21.6% of BTM samples tested positive; true prevalence was estimated as 19.5% of dairy herds based on Se/Sp estimates of 0.9 and 0.95 respectively, with a 95% confidence interval (CI) of 17.5 to 21.5%. This contrasts with the estimated dairy herd prevalence of 38.7% (95% CI 33.1 to 44.3%) reported in a survey which used bulk tank milk samples collected in 2009 (Ryan et al, 2010). An analysis of herd-level risk factors is presented, and the spatial distribution and analysis is displayed in map format.

Conclusions: This survey provides an updated estimate of *Coxiella burnetii* exposure in Irish dairy herds. As the Irish dairy sector is due to expand significantly under the national agricultural strategy (FoodWise 2025), it is essential to monitor any changes in diseases related to this, particularly zoonotic diseases. This study helps to inform policy makers in responding proportionately to concerns in relation to this zoonotic infection of increasing public health importance.

Epidemiology

P03-003-090

Molecular epidemiology of bovine viral diarrhoea virus (BVDV) in Spain between 2007 and 2015, spatial and temporal distribution.

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Objectives: BVDV causes major economic losses in ruminants. Genotypes, BVDV-1 and BVDV-2 have been described. In Europe, BVDV-1 is most frequently isolated. In Spain, BVDV-1b is the most

prevalent subtype, but recently BVDV-2 has been reported for the first time. Studies on the molecular epidemiology of BVDV will provide valuable information about the diversity of the viral strains present in Spain. The objectives of the present study are to: 1) identify the genotypes of BVDV in Spain from 2007-2015; 2) study the spatial and temporal clustering for each genotype, and 3) investigate the trends of diversity of the genotypes along the time.

Materials and Methods: Cattle sera from 164 viremic animals, previously diagnosed by antigen ELISA, were randomly selected from a sera bank collected from 2007 to 2015. For genotyping, a one-step RT-PCR which amplifies a 288 bp of the 5' untranslated region (5'-UTR) previously described was carried out. Products of RT-PCR were sequenced.

The sequences obtained were aligned, and those 100% identical were grouped as nucleotide sequence types (ntST). In order to classify the sequences in the study into different genotypes and subtypes, a maximum likelihood tree with a bootstrapping of 1000 replicates was made with ntST and the reference sequences. In addition, to investigate the spatial and temporal clustering of the sequences, two neighbor-joining networks were constructed, one representing the regions and the other one the years of sampling. For the network analysis all sequences previously described in the Genbank from Spain were also included, all of them being collected from 1999 to 2002.

Results: Main results revealed a high genetic diversity, identifying 88 ntST out of 164 total sequences obtained. All of them belonged to genotype 1. Among subtypes, 1b was the most frequent, representing the 79.9% of total sequences obtained (131/164) and the 77.3% of all ntST (68/88). Other subtypes found were 1f (7.9% total sequences and 11.4% ntST), 1e (6.1% total sequences and 2.3% ntST), 1d (4.3% total sequences and 6.8% ntST), and 1h (1.8% total sequences and 2.3% ntST).

No regional or temporal clustering was observed for each subtype, with the exception of subtype 1h that was only found in one region (Castilla y León) and in one year (2014). In fact, some frequent ntSTs were detected in up to six different regions out of 10 included in the study. Regarding the evolution of the diversity of subtypes, 1b and 1e were found from 1999 to 2015 and from 1999 to 2013, respectively. Eight ntST were present since 1999-2002 to 2007-2015, revealing a certain stability of predominant sequences. Subtypes 1d, 1f and 1h were identified for the first time in 2007, 2008 and 2000, respectively.

Conclusions: The results obtained in the network demonstrate the predominance of subtype 1b in Spain since 1999, which seems to be enzootic. The recent introduction of subtypes 1d and 1f highlights the importance of periodic genotyping of BVD in order to obtain relevant epidemiological information about the evolution of the different subtypes along the time.

Epidemiology

P03-003-091

Epidemiological investigation of prevalence, risk factors and prevailing sequence types of *Brucella* in ruminants of India

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Objectives: Brucellosis is a serious zoonotic disease infecting multiple hosts of land and water. Its taxonomy, host preference and epidemiology are continuously emerging and confusing. In India, 5% decline in national milk production for every five years and loss of 2.63 million female calves per annum with the annual economic loss of US\$ 3.4 billion due to brucellosis have been estimated. The present study was an investigation of prevalence, risk factors and prevailing sequence types in ruminants of India. The study also highlights the comparative analysis of MLST sequences of regional field isolates with that of other Indian and global isolates.

Materials and Methods: Serum samples collected from ruminants of species-cattle, buffalo, sheep and goat were screened by rose bengal plate test (RBPT), indirect enzyme linked immunosorbent assay (iELISA) and bcs₃₁ PCR. Statistical analysis was performed using SPSS software for investigation of risk factors at both animal and herd level. Vaginal discharge and aborted materials were used for isolation. Isolates were analyzed by biochemical and molecular tests (bcs₃₁ genus PCR, AMOS and Bruce ladder PCRs for speciation). Confirmed isolates were sequence typed by multilocus sequence typing (MLST). To determine sequence divergence and phylogenetic relationship among the regional isolates and with other Indian and global isolates, neighbour joining tree was constructed using MEGA 6 software.

Results: A total of 2529 ruminants (Cattle-1233, Buffalo-212, Sheep-719 and Goats-365) were sampled. Among these, 9.43%, 9.32%, 8.06% and 7.12% were declared as seropositive in buffaloes, cattle, sheep and goats respectively. In PCR, 2.08 to 5.6% samples were showed positive amplification in different species of ruminants, thus confirming the active infection status in herds. Ten out of fifteen risk factors analyzed (breed, age, breeding method, history of abortion/ other clinical sign, purchase of new animal, routine diagnosis, veterinary assistance, herd size, hygienic status of farm, education of farm owner and brucellosis awareness) were found statistically significant. A total of thirty field isolates were confirmed by biochemical and molecular tests. MLST typing of field isolates along with reference strains revealed the five distinct sequence types and their spatial distribution in India. B. melitensis isolates of India were typed as ST 7 and ST 8. ST1 found as predominant sequence type among the field isolates of B.abortus from India and among available B. abortus genome sequences from different parts of the world during the period 2000-2015. Also the study underlines the genetic relatedness of the B. abortus S19 vaccine and B. abortus S99 antigenic strains.

Conclusions: The study revealed that organized farms of ruminants in India were significantly affected with brucellosis. Isolation and Brucella DNA in sera, confirmed the active infection status. Thus greater attention needs to be given for brucellosis awareness, pre-purchase testing and routine screening. This is the first attempt to document the sequence diversity of Brucella in India. The knowledge about prevailing sequence types, key routes of infection transmission and risk factors will be helpful to develop suitable control strategies and policies. Also decisive in discriminating between naturally occurring outbreaks and bio terroristic incidences.

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Keywords: brucellosis, India, MLST typing, prevalence, risk factors.

Epidemiology

P03-003-092

Comparative Bacteriological And Molecular Detection Of Group B Streptococcus From Bulk Tank Milk Samples

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Objectives: The aim of this study was to evaluate and compare two diagnostic tests for the detection of Group B Streptococcus (GBS) in bulk tank milk (BTM) samples: The selective chromogenic medium for the screening of GBS (ChromeID[®]) and the molecular Polymerase Chain Reaction (PCR).

Materials and Methods: Ninety-five BTM samples were purposively selected out of a total of 462 GBS isolations. These isolates were previously positive to CAMP reaction in an initial screening. Samples were submitted to the milk quality laboratory of Universidad de Caldas for GBS detection by culture and PCR. All samples were cultured on a Chrome Id[®] media (Biomerieux, Marcy-l'Étoile, Francia), and aerobically incubated at 37 °C for 24-48 h. According to the manufacturer's instruction, presumptive positive colonies for GBS were those observed as red or pink. Chromosomal DNA was isolated from apparent positive and negative colonies using the UltraClean Microbial Isolation Kit[®] (MoBio Laboratories Inc. Carlsbad, United States). Molecular confirmation was done by PCR species-specific, following the methodology reported by Elias et al. (2012). Samples were classified as 0 for negative and 1 for positive to the either test. Considering PCR as the complementary confirmatory test, data were analyzed calculating the sensitivity (Se), specificity (Sp), positive (PPV) and negative (NPV) predictive values for the Chrome ID[®]. The McNemar's test was used to compare the proportion of positive isolations to Chrome ID[®] and PCR tests. Statistical analyses were done in Stata 14[®] (Stata Corp. College Station, TX, USA).

Results: The general prevalence of GBS among herds was 77.8%. However, 68 isolates out of the 95 were confirmed as SGB by PCR (71.6%). Sixty-three isolates were classified as truly positives (66.3%), 3 (3.2%) were truly negatives, 24 (25.3%) were false positives, and five (5.3%) isolates were false negatives. The negative isolates were not molecularly classified into another group of bacteria because it was beyond of the scope of this study. The Se of the chromogenic media was 92.7% (95% IC: 83.7% - 97.6%), while the Sp was 11.1% (95% IC: 2.4% - 29.2%). The PPV was 72.4% (95% IC: 61.8% - 81.5%), and the NPV was 37.5% (95% IC: 8.5% - 75.5%). The McNemar's statistic indicated significant differences (P<0.01) in the proportion of positive isolations between tests; therefore, the Cohen's kappa (k) agreement statistic was not calculated (Dohoo et al., 2009).

Conclusions: Our results showed a significant difference in the proportion of positive samples between both chromogenic media and PCR tests. Despite of the high Se of the chromogenic media (Chrome ID[®]), the Sp of the test was low. These results indicate that the chromogenic media (Chrome ID[®]) can be used as a screening tool for detecting GBS; however, it is important to use a confirmatory test to avoid the false-positive results.

Epidemiology

P03-003-093

Photosensitivity associated with Pithomycotoxicosis in Azores – PortugalClara Dias^{1,1*}Helder Cortes²Carlos Pinto³

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Objectives: Photosensitivity (PTS) is induced by photodynamic agents (PA) circulating in the body, in presence of sunlight, those agents cause damages in skin cells. In Azores, PTS in cattle is mostly induced by Lantana camara and sporidesmin (SPD). SPD intoxication is more common at summer's end and autumn because it's when the sporulation of *Pithomyces chartarum* occurs. The aim of this study is determine the prevalence of cattle, slaughtered at abattoirs of São Miguel and Terceira Islands with skin lesions of photosensitivity (SLP), acute (aPTS) and chronic (hyperkeratosis-HPK), as well as the rates and causes of hepatic and carcasses rejection.

Materials and Methods: To prepare this study, data were collected from abattoirs in São Miguel Island (SMI) and in Terceira Island (TI) between 2008 and 2014 and, between 2005 and 2014, respectively. These data include information about ante mortem inspection (AMI), hepatic rejection (HR) and carcass inspection (CR) of cattle with SLP. Most of the time, data from the abattoirs were held in paper and were transferred to computer support to be worked in a best way. Dubious skin lesions and other skin lesions not linked to PTS and not confirmed by histopathological analyses were not considered.

Results: In abattoir of SMI, between 2008 and 2014, were slaughtered 151,790 cattle, 1,531 (1.01%) with SLP of these 53.76% with aPTS and 43.17% with HPK. October (14.34%) and September (11.79%) were the months with biggest aPTS input at abattoir, while November (11.95%) and October (10.89%) were the same for HPK. 32.2% of the cattle with SLP had their carcasses rejected, 22.99% presenting aPTS, 8.63% HPK and 0.59% both. Carcasses from cattle with aPTS were mostly rejected by acute PTS (35.8%) while in cattle with HPK (34.09%) were rejected mainly by urinary bladder neoplasia. Were rejected 93.54% of the 1,531 livers, 49.12% from cattle with aPTS and 41.48% from cattle with HPK. Independently of AMI and not taking in account the CR causes, the most frequent cause of HR was fibrosis/sclerosis (F/S)–31.03% of the 1,531 cattle. In abattoir of TI, between 2005 and 2014, were slaughtered 157,795 cattle, 751 (0.48%) with SLP of these 56.72% with aPTS and 41.68% with HPK. November and June with 18.31% each one were the months with biggest aPTS input at abattoir, while October (11.82%) and July (10.86%) were the same for HPK. 11.9% of the cattle with SLP had their carcasses rejected, 4.93% presenting aPTS, 6.79% HPK and 0.13% both. Carcasses from cattle with aPTS were mostly rejected by acute PTS (35.14%) while in cattle with HPK (49.02%) were mainly rejected by squamous cell carcinoma. Were rejected 78.3% of the 751 livers, 44.74% from cattle with aPTS and 32.22% from cattle with HPK. Independently of AMI the most frequent cause of HR was F/S–26.9% of the 751 cattle.

Conclusions: Prevalence of cattle with SLP is low, with most of cattle presenting aPTS at AMI. At SMI the months with biggest input numbers at abattoir of cattle with SLP belong to summer's end and autumn, while in IT this is partially true. The CR and HR rates differ a lot in both Islands partially because of lower number of slaughtered cattle at TI and the consequent lower speed of slaughterhouse line, allowing more time for remove improper areas of carcasses for

human consumption. The biggest input of cattle with SLP at abattoir in the specific period of year and F/S as the most frequent HR cause indicate that SPD intoxication can be the origin of PTS.

Epidemiology

P03-003-094

Classification of farm brucellosis status in function of the herd size, test characteristics, and cut-off used.Andres Gil¹Jose Piaggio^{1*}Bruno Lanfranco²Alicia von Gehlen¹Federico Fernandez³

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Objectives: Considering the herd size and the individual serological test characteristics, the goal of this study was to decide what would be the cut-off (number of seropositive animals) which is considered enough evidence to declare a farm as infected.

Materials and Methods: We stratified the farms population according to the susceptible population to analyze potential scenarios. Individual sensibility (Se) and specificity (Sp) was estimated with a simulation model that evaluate the use of 2 test in serial way. Herd specificity (HSp) was estimated for each scenario and different cut-off with the binomial distribution in a simulation model for a combined individual specificity of 0.9999 (0.9923-1.0) to compare the HSp values. Herd sensitivity was also calculate for the same scenarios using the hypergeometric distribution with an individual sensitivity estimated by the simulation of 0.9340 (0.9000-0.9577) and an intra-herd prevalence that ranged from 6% to 1%. A cut-off of 1 will ensure the maximum HSe, but the HSp goes down with the population sizes increase, with the consequent high proportion of false positives.

Results: In the Uruguayans' scenario with national cattle prevalence estimated in 0.15% and farms prevalence estimated in 0.75%, we recommended use different cut-off in function of the herd size. The recommended cut-offs are 1 serum positive cow in farms of up to 300 susceptible cattle (94-98% HSe and 96-100% HSp); 2 positive cows in farms between 301 and 500 (HSe > 89.5% and 98.5-99.6% HSp), and 4 positives cows in farms with more than 500 susceptible animals (95.8-100% HSe and HSp>94.499.8%). In addition, we recommended to no ignoring farms with any serological evidence of brucellosis; the veterinary services should investigated these herds to confirm or rule out the presence of infection.

Conclusions: In conclusion, in farms with large populations a single serum positive is not enough to confirm it as infected without any other evidence of infection. We propose that Legislation and eradication strategy should update with this information.

Epidemiology

P03-003-096

Occurrence of rabies in herbivores from Brazil - 2014Ellen E. Laurindo¹Elaine F. Sena²Joao H. Perotta³Ivan R. Barros Filho^{3,*}

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Objectives: In 1911, rabies transmitted by vampire bats to herbivores was first diagnosed in Brazil. Since 1967 the Ministry of Agriculture, Livestock and Supply (MAPA) establishing national programs to control the disease, in order to reduce its incidence in the country. Among the measures recommended in the program is control of the vampire bat population, vaccinating herbivores and passive surveillance of nervous diseases in herbivores by removal the Central Nervous System (CNS) and referral for diagnosis.

Materials and Methods: In this work, we analyze the Brazilian surveillance system for nervous diseases in herbivores (including cattle, small ruminants and equines), based on collection of data in official and accredited by MAPA laboratories for rabies diagnosis. Additionally, we used data provided by the regional offices of the Official Veterinary Service about species, aging and clinical findings. They were collected and analyzed Brazilian herd cattle data from the herd values indicated in vaccination campaigns for Food and Mouth Disease (FMD).

Results: Brazil has a population of 210,654,620 bovine heads divided into five regions: South (27,161,875), Southeast (38,100,368), Midwest (70,059,177), Northeast (29,179,081) and North (46,154,119). In 2014, 2449 CNS samples of herbivores were sent to laboratories for rabies diagnosis. Of these, 974 (39.7%) were positive for the indirect fluorescent antibody test (IFAT). The region that sent more samples for diagnosis was the Southeast (48.1%), followed by the South (22.5%) and Midwest (12.3%). The regions that showed greater number of positive samples over total positive were the Southeast, South and Midwest with 53.7% (523) 24.2% (236) and 7.1% (69), respectively. Regarding the representativeness of surveillance, it was considered the percentage of cattle in the region in relation to the national cattle herd and the percentage of exams within the total tests performed in the country. Thus, the region with the largest representation was the Southeast (with 18.1% of the national herd and 48.1% of total samples) and South (12.9% of the national herd and 22.5% of total samples). The Midwest, Northeast and North, which are located 145,392,377 head of cattle (representing 69% of the national herd), represented only 29.3% of the samples forwarded for rabies diagnosis,

Conclusions: The surveillance for rabies is carried out in Brazil uneven between regions. Considering the rabies zoonotic character, these differences observed in this study should be considered during the development of new control strategies of the disease in animals and in the protection of human health.

Epidemiology

P03-003-097

Text mining laboratory submission histories to further understand the epidemiology of Theileria associated bovine anaemia (TABA)

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Objectives: Since 2012 New Zealand has suffered a substantial epidemic of infectious bovine anaemia associated with *Theileria orientalis* (Ikeda), a tick-borne obligate intracellular haemoprotazoan

parasite. To date well over 1000 cattle farms in New Zealand have had at least one clinical case of *Theileria* associated bovine anaemia (TABA).

The objectives of this study were to analyse the submission histories for animals naturally infected with *T. orientalis* (Ikeda) and to contrast them for lightly and heavily parasitized cattle, for young and mature animals, for beef and dairy cattle and for severely anaemic, moderately anaemic and non-anaemic animals.

Materials and Methods: The data used in this analysis were collected over 2 years from 17/10/2012 to the 24/11/2014. The histories were taken from the laboratory submission forms for animals that met the case definition for molecular testing. Data were collated from the respective laboratory's submission forms and pre-processed into more precise clinical terminology where appropriate. These included concatenating words so that their context and meaning wasn't lost e.g. cows that were described as losing weight were grouped as "weightloss".

Since the clinical histories were the unit of interest then only one history was used per laboratory submission, even if multiple samples were included in that same laboratory submission. For those submissions that included multiple samples then the average PCV and average piroplasm count was used for categorisation of those histories. In text mining, each of the laboratory submission histories becomes a document in a corpus and from this corpus a term document matrix of the most commonly used words in the histories is prepared after removing unwanted text such as stop words and grammar. The frequency of text was displayed graphically using a word cloud, in which the size of font is proportional to the frequency of text such that the most common words used in the histories have the largest font. For comparison between categories bar plots were used to show the frequency of words with more than 10 occurrences in the histories.

Results: There were 227/281 (80.8%) unique laboratory submissions (276 blood samples) from Ikeda positive cattle, 15/281 (5.3%) from Ikeda negative cattle (19 blood samples) and 39/281 (13.9%) from non-Ikeda positive cattle (54 blood samples). The most commonly used clinical terms in sick Ikeda positive animals were jaundice (58/227), cow (53/227), anaemia (51/227), lethargic (47/227), pale mucous membranes (47/227) and tachycardia (37/227). However there were some marked differences recorded between age groups with jaundice most commonly seen in anaemic adult stock and rarely reported in anaemic young stock. Group problems were almost as frequently reported for calves as individual animals whereas individual sick cows were much more commonly reported for adults. Temperature was also more commonly recorded in young stock than for adults and affected animals were more likely to have normal temperatures than be pyrexia. Ticks were also more frequently recorded on the young stock than for other groups. For the non-anaemic cattle, weight loss, diarrhoea and off milk were most often recorded, with affected animals likely to show ill-thrift.

Conclusions: Practitioner observations are a valuable source of data that is rarely, if ever, used in understanding the epidemiology of a new disease. Methods need to be developed to better capture this data from laboratory submission forms.

Epidemiology

P03-003-098

Association of livestock owner, farm location and livestock movement on FMD in the endemic area with low immunity of animal population

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Objectives: In Lao PDR, FMD is endemic and most farmers are small holders. Although FMD vaccination has been implemented and supported by OIE, a small number of animal population have been vaccinated. To provide a better knowledge of how to control FMD outbreaks and reduce the risk of FMD spread in Lao PDR as the area of low immunity of animal population, the objective of this study is to identify the risks in terms of livestock owners' characteristics, the number of livestock moved, spatial location, density of animal holders and animal population, and outbreak control strategies.

Materials and Methods: FMD outbreak information during 2011-2013 was provided by the Department of Livestock and Fishery, Lao PDR. Using a case-control design, this study employed a questionnaire and interview survey on livestock holders. Information on the important aspect of general information of livestock owners, FMD control and prevention strategies before and after outbreak, FMD emergency vaccination programs during outbreak, farm size, and farm types was captured. The household location and the elevation above the sea level were determined by a GPS tracker (Garmin GPS map 60csx, USA). The network data of animal movement between villages that surveyed in 2012 and matched with our survey villages in this study (29 villages) were analysed. A total of 434 households in 59 villages of 5 districts were interviewed and their data collected comprising 181 case households, 146 control households inside the outbreak villages and 107 control households outside the outbreak villages. Data from questionnaires and spatial data were analysed as independent variables in the logistic regression model using FMD occurrence at the household level as a dependent variable. Network parameters were analysed the association with FMD occurrence at the village level by non-parametric test.

Results: The results show that livestock owners who had knowledge about FMD before the outbreaks were able to better prevent their animals from FMD ($P < 0.01$) although they were less educated and administered less vaccination to their livestock. The livestock owners who were not Buddhist ($P < 0.05$) and younger ($P < 0.05$) in the outside of the outbreak villages were in a lower risk than the owners inside the outbreak villages. The livestock households in the community closer to a main road and lower elevation within a higher number of livestock were at higher risk to developing FMD ($P < 0.01$). Moreover, the information of network parameters at the village level indicated that the villages with high movements of livestock were at high risk of FMD ($P < 0.05$).

Conclusions: In conclusion, this study clearly indicated the association of owners' knowledge about FMD and the community areas with high density of movement on FMD occurrence. The households far away from the centre of community had a decreased probability of FMD occurrence. The owners' awareness is also important to reducing FMD. To prevent FMD effectively in the area with low immunity of animal population, the location of farms should be advised being away from community and livestock owners should be trained and educated in biosecurity to prevent FMD outbreak.

Comments: However, until now, there are still a limited number of livestock that were vaccinated and low levels of biosecurity to prevent FMD within villages. The study to increase immunity and improve the biosecurity at the village level to prevent and control FMD represents a challenging task for future investigation.

Epidemiology

P03-003-099

Study Of The Serological Response Of Bovine Leptospirosis In Cases Of Abortion In Uruguay

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Objectives: Leptospirosis is a bacterial disease caused by pathogens from the genus *Leptospira*. It is a zoonosis of worldwide distribution, that affects most domestic mammals, and wild animals. The disease is considered an occupational high-risk disease for man, who acts as an accidental and terminal host. In cattle, the disease causes great economic losses due to: abortions, infertility, decreased milk production and calves may die during infection. The aim is to study the serological response against leptospirosis in cattle from abortion outbreaks.

Materials and Methods: For the present study the database of samples sent to the Veterinary Laboratories Division of the Ministry of Agriculture and Fisheries (DILAVE) in the period from 2008 to 2014 for the bovine species was analyzed. A total of 10,540 sera samples from 1,483 farms with a history of abortion were analyzed by the microscopic agglutination test (MAT). Sera were checked against 8 serovars (Bratislava, canicola, Grippotyphosa, Icterohaemorrhagiae, Pomona, Hardjo type Hardjoprajitno, Hardjo type hardjobovis and Wolfii). It started with a titer cut-off of 1/200. In positive samples to 1/200, successive dilutions in base of two were carried out until 1/6,400. The data was also stratified by time of year and geographical distribution. The results were analyzed with software STATA 15.0.

Results: The 50.46% of the samples with a history of abortion were positive by MAT starting from a titer of 1/200. Forty seven percent of the farms were seropositive. The data shows that the less reactive serovars throughout the period studied were Canicola, Bratislava, Grippotyphosa and Icterohaemorrhagiae with minor proportions of positive 6% for the four analyzed serovars. For the serovars of the serogroups Pomona and Sejroe (Hardjoprajitno, hardjobovis, Wolfii) you can see a high proportion of positive throughout the study period. For the serovar Pomona an average percentage of 27% was observed, for serogroup Sejroe 32%. It was also demonstrated by the MAT test that there was a 76% of cross reactions between serovars of the serogroup Sejroe. Taking all serovars as a whole, a homogeneous distribution is observed in the studied years, noticing a relative increase in 2009 and a reduction in 2014. There was no difference between the seasons. The geographical distribution was 57-59% for North, South and West and less than 45% for the eastern region of the country.

Conclusions: The data analyzed in the study period (2008-2014) shows that the serovars with more presence with a serological response in the cattle population of Uruguay are: Hardjo with his two biotypes. Hardjobovis and Hardjoprajitno, Wolfii and Pomona. The variations between years can be explained by climate and seasonal influence of the disease. Uruguay disease occurs throughout the year. It is endemic, the annual incidence may vary depending on the weather. MAT is a good test to diagnose positive farms with history of abortions to Leptospirosis.

Epidemiology

P03-003-100

Comparative analysis of Brazil and USA bovine spongiform encephalopathy (BSE) national monitoring systems and the challenge of atypical BSE

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Objectives: Although the Bovine Spongiform Encephalopathy (BSE) is under control, other forms of the disease have been reported, initially in Europe, and the knowledge about their impact on public health remains uncertain. In compliance with this importance in international trade, the World Animal Health Organization recommends actions aimed at mitigating the risk of spread the BSE around the world. Brazil and the United States are countries with large commercial cattle herds, and in both there was only the occurrence of atypical BSE indigenous cases.

Materials and Methods: In this work, the BSE monitoring systems were analyzed in these countries, based on regulatory laws, legal support of health programs, surveillance strategies and emergency plans of the official veterinarian services and this data. They were also evaluated and compared them to the animal health care systems, including laboratory capacity, participation of veterinarians from the private sector in animal health protection actions and number of veterinary medicine educational institutions of each country.

Results: We founded that both systems meet the guidelines of the World Organization for Animal Health. However, this same organization does not call for actions to prevent or control that specifically address the atypical BSE, as the target of the surveillance are ruminants with the presentation of the classic picture of the disease. In this sense, is necessary the reassessment of priorities and give more importance to all other surveillance categories (older and fallen bovines and those destined to abattoirs).

Conclusions: Regarding the Brazilian surveillance system, it can be concluded that there is need for profound changes in the packaging system samples and laboratory tests, because the current system is focused on the diagnosis of classical form of BSE. We concluded that the BSE monitoring system in Brazil is more focused on animal health, while the US system is aimed the meat consumer protection. The adjustments identified in this work are needed for Brazilian system reaches a level of excellence and to provide more security for consumers of Brazilian meat products.

Objectives: Bovine Respiratory Diseases (BRD) are a major pathological complex in cattle, with significant impact on farm economical results. The pathogen agents responsible for these disorders are essentially viruses and bacteria, often associated on the field. Polymerase Chain Reaction (PCR) is a major technique of diagnosis, due to its better detection performance in comparison with traditional bacteriological methods. The objective of this study was to determine the field prevalence of BRD pathogens using the PCR technique in France.

Materials and Methods: Since December 2012, 2 major laboratories for diagnosis located respectively in Nièvre and Ile et Vilaine departments participated in this field BRD epidemiological survey, with support from MSD Animal Health. The sampled animals, from a few days to several months old, had generally clinical symptoms of respiratory disease in early evolution. After sampling by the vets either from deep nasal swabs (3 swabs per farm) (80% of all samples) or trans-tracheal fluids (<10%) or pulmonary tissues of animals, PCR was performed after mixing maximum 3 samples from the same BRD outbreak in the same farm. A total of 648 individual viral and bacterial diagnosis were analyzed.

Results: In the present study, a grand majority of the analysis showed the presence of bacteria with more than 80% of the samples positive for *Pasteurella multocida* (up to 85% associated with another pathogen) and 46% of samples positive to *Mannheimia haemolytica*. In the other hand, *Histophilus somni* and *Mycoplasma bovis* were found to a lesser degree. Regarding BRD viruses, Bovine Respiratory Syncytial Virus (BRSV) was detected in less than one third of all cases, half in association with *M. haemolytica*.

Conclusions: This prevalence study in French field farms confirmed the presence of the 2 major BRD pathogens: 1) *M. haemolytica*, for which one could think that it could be overestimated because of the sampling technique (nasal swabs) and the testing method (PCR). But former studies showed a correlation between nasal swabs and trans-tracheal aspiration on pooled samples (3 particularly), and 2) BRSV, for which the prevalence of 30% could seem to be low.

Epidemiology

P03-003-101

Field prevalence of bovine respiratory pathogens by PCR technique in France

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Genetics and Breeding

P04-004-027

Underlying mutation(s) for a deformed cleft pallet in a Holstein-Friesian sire familyMichelle Judge¹ Brian Kirkpatrick² Donagh Berry¹¹Teagasc, Fermoy, Co. Cork, Ireland, ²University of Wisconsin-Madison, Madison, Wisconsin, United States

Objectives: A deformed cleft pallet phenotype was detected among a small proportion of the offspring of a phenotypically normal Holstein-Friesian bull. The objective of the present study was to test the hypothesis of a single gene controlling the phenotype and to map the gene.

Materials and Methods: Pedigree analysis of affected calves was undertaken. Bovine50 Beadchip genotypes (i.e., 54,001 single nucleotide polymorphisms; SNPs) were available on the phenotypically normal Holstein-Friesian sire and high density genotypes (i.e., 777,962 SNPs) were available on both his affected (n=23) non-affected (n=44) paternal half-sib progeny. The genotypes of each individual were phased, and were subsequently used to predict paternally inherited haplotypes in the offspring. The hypothesis of segregation of a single gene was tested and mapping of the gene was performed by linkage analysis using two alternative approaches. In the first approach, the association of the paternally inherited haplotype with the affected phenotype utilised both affected and non-affected offspring (n=67) in a typical linkage analysis. In the second approach, only offspring with the affected phenotype were used (n=23), thus testing the deviation of the paternal haplotype inheritance from the expectation of equal representation of alternative haplotypes under the null hypothesis of no association.

Results: Affected calves did not display obvious inbreeding to a common ancestor thus essentially ruling out the likelihood of a recessive mutation being the likely causative factor. Linkage analysis results, based on all offspring, resulted in significant associations for regions on both distal BTA13 ($p < 2 \times 10^{-5}$) and BTA17 ($p < 2 \times 10^{-4}$). When only including affected offspring in linkage analysis, the significance of the association observed on BTA13 increased considerably ($p < 1 \times 10^{-24}$) and became more obvious than on BTA17.

Conclusions: Affected calves did not display a common ancestor suggesting that the causative allele was not a rare recessive. The only commonality among pedigrees was the sire, and his normal phenotype suggests that either incomplete dominance of an allele or a mosaic mutation are the likely modes of gene action; the mutation(s) appears to reside on distal BTA13. Whole genome sequence of two affected calves will be undertaken in future investigations.

Genetics and Breeding

P04-004-028

Sire prevalence of Bovine Viral Diarrhoea infection in Irish cattleSiobhán C. Ring^{1,2} Michael L. Doherty² David A. Graham³ Margaret M. Kelleher⁴ Donagh P. Berry¹¹Animal & Grassland Research and Innovation Centre, Teagasc, Moorepark, Co. Cork, ²School of Veterinary Medicine, UCD, Belfield, Dublin 4, ³Animal Health Ireland, Main Street, Carrick on Shannon, Leitrim, ⁴Irish Cattle Breeding Federation, Highfield House, Bandon, Co. Cork, Ireland

Objectives: Bovine viral diarrhoea (BVD) is one of the most important infectious diseases affecting the productivity and international competitiveness of the Irish livestock industry. Production losses associated with BVD cost dairy and beef producers an estimated €102 m per annum. The objective of this study was to quantify the contribution of genetics to differences in resistance to BVD in Irish dairy and beef cattle. Results will be useful in determining the feasibility of genetic selection of cattle for increased resistance to BVD.

Materials and Methods: BVD virus test results from the national BVD eradication programme were available for 4,519,810 calves in 82,585 herds between the years 2012 and 2015, inclusive. BVD tests were undertaken using either an enzyme-linked immunosorbent assay or reverse transcription polymerase chain reaction test. Test results were classified as negative or positive according to the kit manufacturers' guidelines. Animals were defined as persistently infected (PI) or non-persistently infected (non-PI) based on either an animal's own test result(s) or its progeny/dam test result(s). To maximize the likelihood of equal lifetime exposure to BVD virus between herd-mates, only dams that remained in the herd since birth, with known BVD infection status (i.e., PI or non-PI) for the dam and her progeny were retained. Exposure of a dam and her progeny to BVD virus was defined by the birth of a contemporary PI calf/calves; this was undertaken for primiparous and multiparous cows separately. Only data from singleton calves with a known sire in exposure groups with ≥ 5 calves were retained. After all data edits, 79,618 calves from 3,530 exposure groups in 2,953 herds remained. The mean prevalence of BVD in the progeny of individual sires was estimated for sires with > 50 progeny in > 10 herds. Also, the mean prevalence of BVD in the grand-progeny of individual maternal grandsires was estimated for maternal grandsires, that produced non-PI female offspring with > 50 grand-progeny in > 10 herds. The heritability of BVD is currently being estimated.

Results: The overall prevalence of PI calves in the final dataset was 5.83%; this is not indicative of the national population as the dataset was edited to only include animals with maximum potential of exposure to BVD virus; the prevalence of PI calves in this study prior to elimination of animals not exposed to BVD virus was 0.36%. The mean prevalence of PI calves per sire (n=171) ranged from 0 to 0.23. This indicates that some sires did not produce PI offspring, while other bulls sired up to 23% PI offspring; all calves were born in close proximity in time to a contemporary PI calf. Furthermore, the mean prevalence of PI calves per maternal grandsire (n=191) ranged from 0 to 0.15. This indicates that some maternal grandsires did not produce any PI grand-progeny, while other grandsires produced up to 15% PI grand-progeny.

Conclusions: This preliminary study indicates that the prevalence of BVD PI calves differs between paternal half-sibs as well as among grand-progeny indicating underlying genetic variability in susceptibility to BVD. Heritability estimates will aid in quantifying this genetic variation.

Genetics and Breeding

P04-004-029

Chronic diarrhea associated with Cholesterol Deficiency in Holstein Calves: A Case SeriesThomas Mock¹ Kemal Mehinagic² Fiona Menzi³ Cord Droegemueller³ Mireille Meylan¹ Nadine Regenscheit²¹Clinic for Ruminants, ²Institute of Animal Pathology, ³Institute of Genetics, Vetsuisse Faculty, University of Berne, 3012 Bern, Switzerland

Objectives: A new inherited autosomal recessive genetic defect in Holstein calves, named cholesterol deficiency (CD), has been reported recently in Germany. The Canadian sire MAUGHLIN STORM has been detected as the first carrier bull. Homozygous calves show clinical signs of diarrhea unresponsive to treatment and failure to thrive, have very low blood cholesterol and triglyceride concentrations, and usually die within the first six months of life. The aim of the present study is to describe the clinical picture, necropsy findings and the genetic defect causing this new disease entity.

Materials and Methods: Five calves with a history of failure to thrive and intermittent diarrhea unresponsive to treatment were referred to the Clinic for Ruminants of the Vetsuisse Faculty in Berne. A full clinical examination and measurements of cholesterol and triglycerides were performed for all calves, as well as a complete blood count and blood chemistry profile for three calves. Faecal samples were sent for viral, bacterial and parasitological analysis for the most common pathogens causing calf diarrhea (rotavirus, coronavirus, cryptosporidia, coccidia, *E. coli* and *Salmonella* spp). The five calves were euthanized and immediately necropsied. Macroscopical and histological examinations were performed. A recently developed PCR-based direct gene test was applied to determine the genotype using genomic DNA extracted from EDTA blood samples.

Results: All five affected Holstein calves were inbred to the assumed founder MAUGHLIN STORM. The mean age of the calves was 86 days (min-max: 18-224 days). All calves were poorly developed and underweight, but otherwise in a good to moderately reduced general condition, mostly with a good appetite. They showed intermittently soft to liquid faeces. Physical examination was otherwise without abnormalities. The concentrations of cholesterol (mean: 0.19 mmol/L, min-max: 0.09-0.24 mmol/L) and triglycerides (mean: 0.04 mmol/L; min-max: 0.04-0.06 mmol/L) were distinctly below the values obtained from five healthy Holstein controls of comparable age (min-max: cholesterol: 1.79-3.20 mmol/L; triglycerides: 0.27-0.43 mmol/L). The blood work of one calf showed additionally a slight anaemia and a low selenium concentration. Coprological examination revealed the presence of *Cryptosporidium parvum* and *Eimeria bovis* oocysts in one calf each. At necropsy, the small and large intestines were filled with liquid, partially foamy and mucinous content, and the wall of the small intestine appeared diffusely oedematous. Histological examination of the small intestine revealed enterocytes containing large amounts of optically empty, intracytoplasmic vacuoles, ranging from 2-20 µm in diameter which stained positive in the Sudan stain for lipids. The genetic analysis revealed that all calves were homozygous for the responsible APOB (apolipoprotein B) gene mutation shown to be associated with cholesterol deficiency.

Conclusions: The clinical presentation and necropsy findings associated with CD had not been described in detail to date. A clinical suspicion in Holstein calves can be confirmed using the new gene test. This new diagnostic tool will allow for a reduction of unnecessary costs of treatment, use of antibiotics and time-consuming care of affected animals, and targeted eradication of the causative mutation from the Holstein population. Since the frequency of the deleterious mutation in the global Holstein population is supposed to be high, this disease has a significant impact on the rearing success of calves.

Genetics and Breeding

P04-004-030

Targeted DNA sequencing of the expanded β-defensin gene repertoire in bulls of high and low fertility

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Objectives: Host Defence Peptides, specifically β-defensins, have been shown to influence fertility across a range of mammalian species, including in mice and in men. However, association between genetic variation in the expanded suite of 57 bovine defensin genes and fertility in bulls has not been previously investigated. Building on our discovery of a novel β-defensin gene cluster in the bovine genome, we used a targeted DNA sequencing approach to capture diversity at all known defensins in bulls of known fertility status used for artificial insemination (AI) in Ireland.

Materials and Methods: Bulls divergent in pregnancy rate data (>1000 AI records) were identified and selected for targeted DNA sequencing. DNA baits were designed to capture complete gene sequences plus 1Kb upstream, covering 235 kb of the 387 kb β-defensin gene clusters genes, when repetitive regions were excluded. DNA was extracted from sperm of 144 bulls representing the extremes of fertility, libraries prepared using Illumina TruSeq, captured with the Nimblegen SeqCap EZSeq custom capture and sequenced on a MiSeq. Reads were aligned to the reference genome and variants identified following the GATK Best Practice pipeline and quality filtered. An association study between the bull fertility phenotype using an adjusted animal model and the variant genotypes was performed.

Results: Targeted regions had an average coverage of 197 X. Following editing and quality control, 2836 SNPs were examined, 37% of which are novel and have not been previously described in cattle. 7.5% of SNPs are found in gene exons, and 25%, 23% and 22.5% are upstream, intronic, or downstream, respectively. 702 SNPs were breed specific and some chromosomal regions show runs of breed-specific variants. A haplotype containing 94 SNPs covering ~138kb and found in 9 highly fertile bulls was found to be significantly associated with fertility. Functional assessment of these SNPs in sperm from high and low fertility bulls is currently ongoing.

Conclusions: This is the first comprehensive analysis of sequence variation present in these bovine genes which play critical roles in immunity and fertility. A targeted sequencing discovery of β-defensin diversity in bulls used in AI identified a haplotype spanning a region containing several β-defensin genes associated with fertility. The region includes the bovine homologue of defensin 126 which has been shown to play a role in male fertility in humans, macaques and rodents. These SNPs have been added to the International Beef and Dairy SNP chip to validate their association with fertility (and potentially other) phenotypes of economic interest.

Genetics and Breeding

P04-004-031

Landscape geography of locally adapted sheep in Brazil using SNP markers

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Objectives: Genetic landscape describe and explain how the landscape and its components affect the genetic variation of populations animals and plants. The goal of this investigation was to explore patterns of spatial genetic structure in locally adapted sheep breeds in Brazil.

Materials and Methods: A total of 215 individuals from eight different locally adapted Brazilian breeds were genotyped using the 50KSheepSNPChip. All samples were georeferenced and genotyped with the Illumina Ovine SNP50 BeadChip. Data quality control and pruning were performed with Golden Helix SVS v7. Markers with call rates <98% and minor allele frequency (MAF) values <0.1 were removed. Non-autosomal markers and all markers in linkage disequilibrium (using r^2 greater than 0.05 (indep-pairwise 50: 5: 0.05) were excluded using Plink. The window size in SNPs was 50, the number of SNPs to shift the window at each step was 5 and 0.05 represents the r^2 threshold. The final dataset contained 23,613 SNPs.

All data sets were initially analyzed via Mantel tests and spatial autocorrelation as implemented in Alleles In Space (AIS; Miller, 2005). In Mantel tests, 5000 randomization replicates were used to evaluate the significance of correlations between $p/(1-p)$ or $D/(1-D)$ and the natural logarithm of geographic distance. For spatial autocorrelation analyses, distances were varied from 5 to 20 classes. Data were analyzed using the Genetic Landscape Shape procedure in AIS. This procedure yields an interpolation-based graphical depiction of spatial genetic structure and diversity across landscapes that can be used to identify genetic discontinuities or landscape regions where relatively high or low genetic distances exist. The procedure was performed by initially generating a Delaunay triangulation-based connectivity network for each species' set of sampling locations and 1000 permutations performed.

Results: The correlation of genetic and geographical distances using the Mantel test was -.0485. The probability of observing a correlation greater than or equal to observed was $P = .873$ and less than or equal to observed = .127. This is probably due to high individual variation within breeds and locations sampled.

The average pairwise genetic distance from the full data set was 0.567 using the Spatial Autocorrelation Test. From the analysis of full distogram: $V = .00136$ with a probability of observing a random value of $V \geq$ observed V by chance had a $P = .25800$. The Allelic Aggregate Index Analysis showed no clear pattern in genetic discontinuity with sheep breeds reared in Brazil with a low number of classes, but with higher number of classes, a tendency for genetic discontinuities between 188 and 938km was noticed.

The interpolation of the Genetic Landscape Shape shows high genetic discontinuities in the northeast and south of the country

Monmonier Maximum Distance Algorithm results indicates an initial subdivision separating the Crioula Lanada from Santa Catarina from the others and then the Pantaneira population from the others. Further subdivision separates hair sheep in Northeastern region including the states of Ceara, Paraiba and Maranhão with the breeds Morada Nova, Somalis Brasileiro and Santa Ines. Little differentiation is seen in the Centerwest region, which may indicate that although samples collected were from purebred animals, these may have already suffered some level of miscegenation as sheep breeding in this region is relatively recent.

Conclusions: High genetic differentiation is seen in the Northeast and Southern regions of Brazil. The results indicate a minimum distance of 188km between collection sites for future sampling, including the Southeast region.

Genetics and Breeding

P04-004-032

Defensin Gene Variation and Fertility in Irish Bulls

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Objectives: To address fertility in the Irish national herd, the focus, in literature, to date has been on female fertility traits. In Holstein-Friesian, pregnancy rates can fall as low as 25% with low fertility bulls, yet no single diagnostic test can accurately predict fertility in bulls, thereby limiting genetic gain for male fertility. Mutations in β -defensin genes have been associated with fertility in multiple species including humans, but have not previously been investigated in cattle. In order to identify genetic variants affecting male fertility, whole-exome sequencing and targeted β -defensin gene sequencing of Irish AI bulls was performed.

Materials and Methods: Bulls divergent in Pregnancy Rate (PR) data and an adjusted animal model (a PR model which also includes environmental factors), were identified and 24 bulls with extreme fertility were selected for sequencing. DNA baits were custom-designed to capture all exons annotated in the bovine genome (plus 100bp of 5'UTR) as well as complete β -defensin gene sequences (plus 1kb of 5'UTR). DNA was extracted and libraries were prepared with Illumina TruSeq Nano kits. DNA was captured using Nimblegen's SeqCap EZ developer kit, and sequenced on a HiSeq2500 for exome libraries, and MiSeq for β -defensin libraries. Reads were quality filtered, aligned to the bovine UMD3.1 reference genome, and genetic variants were called via GATK's HaplotypeCaller, following the best practice pipeline. Variants were annotated and an association of variants with the adjusted animal model phenotype was performed.

Results: Mean target coverage of whole-exome regions was 18.5X, with 95% being covered at 2X depth and 69% of the exome covered at a depth of 10X. Variant calling identified 258,870 SNPs, 12,124 insertions and 13,048 deletions, following variant filtration in 24 bulls. Of these, 38% were located within exons and 2.5% were located within the 5'UTR. Of the exon mutations, 47% were non-synonymous, 43% synonymous and 1.6% resulted in a frameshift mutation. Variants with a frequency difference >25% and which had a call rate of at least 80% were plotted, resulting in 3,314 variants divergent between high and low fertility bulls. Association analysis via the R bioconductor package, GenABEL, identified 405 SNPs significantly associated with fertility phenotype (unadjusted $P < 0.01$). Of the top 20 most significantly associated SNPs, 6 were non-synonymous, 5 synonymous, 1 stop gained, and 8 were located in regulatory regions upstream and downstream. The SNP which was most significantly associated with the adjusted animal model phenotype was located in the 3'UTR of the Progesterone Receptor Membrane Component 1 gene, PGRMC1, the human homolog of which functions in steroid signalling, p450 activation and drug metabolism.

Conclusions: In conclusion, 3,314 variants have been identified in AI bulls with extreme deviation for Pregnancy Rate. 405 variants have been associated with an adjusted animal model fertility phenotype and from these variants, discovered in bulls of divergent phenotypes, 669 have been added to the International Dairy and Beef SNP chip (v3) used for national cattle genotyping and will determine their association with fertility and health phenotypes in large numbers of independent samples.

Genetics and Breeding

P04-004-033

Geographical distribution of zebu breeds in Brazil and their relation to climatic, environmental factors

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Objectives: The objective of the present study was to evaluate the distribution of different zebu breeds in Brazil, correlating their occurrence with climate variables, environmental and human development indicators.

Materials and Methods: The location of all pure herds of zebu cattle in Brazil were made available the ABCZ (Brazilian Association of Zebu Breeders), classified as meat type (Brahman, Polled Brahman, Nelore, Polled Nelore, imported Nelore and Tabapuã), milk type (Gir and Polled Gir), and dual purpose (Guzerá, Indubrasil, Polled Indubrasil, Red Sindi and Polled Red Sindi) being spacialized in ArcGIS® program along with climate conditions (precipitation, Normalized Difference Vegetation Index - NDVI, relative humidity, temperature, Temperature Humidity Index - THI), environmental (altitude, rivers and streams protected by forests, rivers and streams without forest protection, establishments with and without family agriculture, areas planted with forage, degraded planted pastures, planted grazing areas in good condition, areas with agrosilvopastoral systems, areas with rotational pasture handling), and human development indicators (Human development Index - longevity and Human Development Index - education). The statistical analysis included an analyses of variance and logistic regression in SAS® v.9.3 software.

Results: The highest concentration of zebu animals is in the Midwest region of Brazil, followed by the Southeast and North, where Pará state stands out. The South region there was a lower frequency of zebu breeds as these states traditionally use European breeds. In the states in the Northeast the lower distribution of zebu cattle in the region compared to other locations is probably due to its extreme climate, highly susceptible to long periods of high temperatures and absence of rainfall, which directly affects local livestock. The meat breeds were evenly spread throughout the country, probably due to them fitness for meat production, in particular the Nelore. The meat breeds present differed due to environmental variables, showing a higher incidence with increased rainfall, NDVI, temperature, relative humidity, THI and solar radiation, as well as the environmental variables: establishments without family agriculture and rivers and streams protected forests. The dual purpose and dairy breeds, showed significant effects of areas planted with forage, agrosilvopastoral systems and rotational pasture management, indicating a dependency of dairy production on crops for nutrition to support milk yield. The Gir breed, the only with exclusive dairy exploration, showed herds in regions with higher family agriculture, characterized by small to medium farms, and in regions with higher altitude.

Conclusions: The geographical distribution of the various zebu breeds may contribute to better understanding of their adaptation to the environment, assisting in the development of environmental indices, possible corrections of production management, genetic evaluations, breed choice for certain environments, among others.

Genetics and Breeding

P04-004-034

Genetic parameters for *Fasciola hepatica* infection in dairy and beef cows

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Objectives: *Fasciola hepatica* (liver fluke) is a helminth parasite of economic importance to the cattle industry globally. A high prevalence of *F. hepatica* has been reported in herds across the world. Genetic variability for a range of cattle diseases is known to exist. However, to our knowledge, no genetic parameter estimates exist for susceptibility to *F. hepatica* in cattle. The objective of this study was to estimate genetic parameters for susceptibility to *F. hepatica* infection in Irish dairy and beef cattle.

Materials and Methods: In Ireland, cattle livers are inspected for *F. hepatica* at slaughter. Damaged livers are diagnosed as either "live *F. hepatica* observed" or "*F. hepatica* damaged without the presence of live *F. hepatica*". Only animals with a high likelihood of exposure to *F. hepatica* infection were considered. Cows were deemed to have a high likelihood of exposure if they resided in a herd within a period 100 days flanking the date of slaughter of a cow herd-mate with recorded live *F. hepatica* at slaughter. When a cow with *F. hepatica* damage without the presence of live *F. hepatica* was slaughtered, herd-mates that were born into that herd within 100 days of that cow, and remained within that herd until at least 100 days prior to the diagnosis of that herd-mate, were also assumed exposed. Cows deemed exposed, with a known sire and no herd movement after 90 days of age were retained. Contemporary groups of herd-year-season of final calving date of <4 cows were discarded. The final dataset consisted of 16,734 cows from 2,865 contemporary groups. (Co)variance components for liver fluke were estimated using linear mixed models. Fixed effects included in the model were contemporary group, heterosis, recombination loss, parity, age within parity, and abattoir by slaughter date. Random effects were animal genetic effects and residual effect. The pedigree of each animal in the analysis was traced back to the founder population which was allocated to genetic groups. The mean prevalence of *F. hepatica* liver damage per paternal half-sib group was quantified for sires with ≥ 30 progeny in ≥ 10 herds.

Results: The prevalence of *F. hepatica* damage in 16,734 cows included in the analysis was 47%; this should not be taken to represent a national statistic as the data were highly edited for genetic analysis. The heritability (standard error in parenthesis) of *F. hepatica* liver damage was 0.028 (0.0117). The genetic standard deviation for *F. hepatica* liver damage was 0.068 units. The mean prevalence of *F. hepatica* liver damage per sire (n=78) ranged from 14% to 71%. This signifies that some sires had only 14% of their progeny positive for *F. hepatica* liver damage while other sires had 71% of their progeny positive for *F. hepatica* liver damage

Conclusions: Heritable genetic variation exists in susceptibility to *F. hepatica* liver damage. *F. hepatica* liver damage is routinely diagnosed in the majority of Irish abattoirs; therefore national genetic evaluations for *F. hepatica* susceptibility are possible.

Genetics and Breeding

P04-004-035

Geographical evaluation of the Brazilian genetic resource conservation programConcepta Mcmanus¹ Potira M. Hermuche¹ Luiz Felipe Ramos Carvalho² Bruno S. L. Dallago¹ José L. Jivago¹ Samuel R. Paiva³¹University of Brasilia, ²Ministerio da Agricultura Pecuaria e Abastecimento, ³Embrapa Genetic Resources and Biotechnology, Brasilia, Brazil**Objectives:** The aim of this work was validate the integration of spatial (georeferenced) and genetic data regarding local of origin from 3518 DNA samples from 17 different genetic groups or breeds of sheep in the Brazilian Germplasm bank**Materials and Methods:** Samples of sheep in the Germplasm Bank of Embrapa Genetic Resources and Biotechnology was geo-referenced using a geographic information system (software ArcGIS 9.3).

DNA was extracted from 383 individuals and 10 breeds and quantified by spectrophotometry. 22 microsatellite markers were optimized: OarFCB20, ILSTS05, OarFCB48, ILSTS11, ILSTS87, INRA35, INRA05, INRA63, OarAE129, OarFCB304, OMHC1, OarHH35, OarJMP29, INRA23, MAF65, MAF214, BM827, HUIJ616, OarCP20, INRABERN172, SRCSP05, BM6526 were chosen due to their availability and were on the list recommended by FAO

Genotyping including intra-breed and intra-populational variability parameters such as number of effective alleles per locus, allelic diversity, number of diagnostic (specific) alleles per breed, observed (H_o) and expected (H_e) heterozygosity under Hardy-Weinberg (EHW) equilibrium, polymorphic information content (PIC) and fixation index (F_{is}) or inbreeding coefficient.

Distances between DNA collection site and the conservation nucleus responsible for the collection were calculated and submitted to an analysis of variance to see if there was a difference in the distances per breed and per conservation nucleus.

Results: Data from the analysis of molecular variance (AMOVA) show that 12.87% of total observed variance ($p < 0.001$) was due to inter-breed differences. Intra-breed variability showed that the Santa Inês breed had highest heterozygosity values as well as mean number of alleles per locus (0.7580 and 8.39 respectively) while the Somali and Corriedale breeds had the lowest values for these indices respectively (0.5752 and 4.22). With the exception of the Hampshire, all breeds had inbreeding coefficients (F_{is}) less than 10%.

Inter-breed results and AMOVA suggest small genetic differences between sheep breeds in Brazil. The structure analysis shows good separation of the different breeds with subdivisions in the Santa Inês and Morada Nova breeds. The mean genetic distance between pairs in the full data bank using microsatellite data was 0.67 and was not affected by the geographical distance between the sampling sites, independent of breed.

The group of data showed a Moran index (spatial autocorrelation) of -0.063. The correlogram shows seven distance classes (in degrees) from the northern most point (OMN_VE collected in Sobral, Ceará state). The autocorrelation is low with a high coefficient of variation. This shows high diversity between samples, even those that are collected close to each other. Therefore they may be considered independent samples, emphasizing the unicity of the breeds. The spatial autocorrelation between geographic and genetic distance is low, i.e. although they are spatially close, the breeds are genetically distinct from each other.

Conclusions: The Animal Germplasm Bank has been fulfilling its role

of storing the genetic material of endangered breeds. There is need to update the core conservation as well as to collect samples at other locations outside the core for some breeds. Information is needed on the distribution of animals by breed in the country to improve and consolidate the sampling germplasm bank with national coverage for sheep in Brazil.

Genetics and Breeding

P04-004-036

Effect of β -defensin 126 haplotypes on the ability of bovine sperm to bind to bovine oviductal epithelial cells (BOEC) in vitroAlan Lyons¹ Emma Finlay² Pat Lonergan³ Kieran G. Meade² Sean Fair⁴¹Department of Life Sciences, Faculty of Science and Engineering, University of Limerick, Limerick, ²Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Meath, ³School of Agriculture and Food Science, DCU, Dublin, ⁴Department of Life Sciences, University of Limerick, Limerick, Ireland**Objectives:** We have recently discovered a cluster of 19 novel β -defensin genes, some of which are exclusively expressed in the epididymis of the bull and in the oviduct of the cow. Previous studies in other species (humans, macaques and rodents) have suggested a role for β -defensins in mediating sperm function and/or immune-protection of the sperm in the female tract and thus they are likely to play a key role in the successful establishment of pregnancy. The objective of this study was to functionally characterize the relationship between β -defensin 126 haplotype in bulls and the ability of sperm to bind to bovine oviductal cells (BOEC) in vitro.**Materials and Methods:** Adjusted bull fertility phenotypes (based on a minimum of 1000 inseminations) for 7000 AI bulls were used to identify bulls of high and low fertility. The most divergent bulls ($n=150$) were selected for targeted sequencing of β -defensin genes and an association study was performed to identify genetic regions significantly associated with male fertility. The most significantly associated single nucleotide polymorphisms (SNPs) were located in a haplotype consisting of 94 SNPs over 138kb, including the β -defensin 126 gene, found in bulls of high fertility. To examine the effect of the haplotype on BOEC binding, sperm from bulls with and without the β -defensin haplotype from both high and low fertility groups were compared. Briefly, BOEC were isolated from heifer oviducts, immediately post-mortem and seeded onto Merck Millipore EZ slides (Merck Millipore, Cork, Ireland) and cultured for seven days until a confluent monolayer was formed. Hoechst 33342-stained sperm from high fertility bulls with (H+ive; $n=3$) and without (H-ive; $n=3$) the haplotype as well low fertility bulls without (L-ive; $n=3$) were added to the cell monolayers for 30 min. Loosely bound sperm were gently washed away and the culture was then fixed with 2.5% glutaraldehyde. Sperm were observed under half-light/half fluorescence at 40X and the number of bound sperm was assessed by counting 10 random fields of view for each well. Data were analysed for normality of distribution, transformed where appropriate and analysed using analysis of variance in SPSS software. All results are presented as mean \pm s.e.m.**Results:** The mean adjusted pregnancy rate for the H+ive, H-ive and L-ive treatments was +4.3%, +5.4% and -6.7%, respectively. Preliminary results demonstrate no effect of the average number of sperm bound per field of view (4.9 ± 1.32 , 4.8 ± 1.47 and 4.8 ± 1.38 for the H+ive, H-ive and L-ive treatments, respectively). Sperm motility as assessed using computer assisted sperm analysis (CASA) was 55.3 ± 11.90 , 50.9 ± 10.52 and $38.2 \pm 4.49\%$, for the H+ive, H-ive and L-ive, respectively

($P > 0.05$). Similarly, there was no effect of treatment on any of the other post-thaw sperm motility or kinematic parameters assessed.

Conclusions: Our preliminary findings suggest that sperm from bulls of different β -defensin 126 haplotypes, and which differ in phenotypic fertility, do not differ in their post-thaw motility, kinematic or sperm binding properties. Fertility is a complex and multi factorial process and β -defensins may play a role in other aspects of fertility such as mucus penetration and fertilising ability.

Genetics and Breeding

P04-004-037

Economic values for production, functional and fertility traits in milk production systems in Southern Brazil

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Objectives: The objective of this study was to predict the economic values for production, functional and fertility traits, as well as to rank them in order of economic importance for milk production systems in southern Brazil.

Materials and Methods: On farm data were collected by technicians of the Brazilian Sebrae, through monthly monitoring of husbandry and economic data on 61 properties belonging to the microregion of Passo Fundo, southern Brazil, between 2009 and 2011. Two clusters were formed according to their production and the characteristics considered were: milk production (MP), lactation persistency (LP), milk fat (FAT), milk protein (PROT), somatic cell count (SCC), age at first calving (AFC), calving interval (CI), feed intake (Intake), mortality (MORT) and cow weight. The herd size was standardized to a 100-cow farm. The FACTOR procedure was used to understand the correlation structure and the sources of data variation. Analyses of variance were performed for economic and related milk production variables to differentiate between the clusters formed. Clusters 1 and 2 were designated high and medium-high output respectively, used for the economic values calculation. Revenue was based on the sale of milk, surplus heifers and cull cows and the actual operating costs were used to calculate profit and gross margin. The profit function used to calculate the economic values of traits related to milk quality was based on a 100-cow farm in three different scenarios (company that do not bonus/penalize for milk quality (I), which bonus or penalizes the lower range (II) and the higher range (III)). Sensitivity analyzes were performed to assess the impact of possible changes in the variations of dietary components in ± 25 and $\pm 50\%$ on the profit, when there is an increase of 1% in expression of the traits examined.

Results: The high production system (cluster 1) had higher production ($P < 0.10$) than the medium-high production system (cluster 2) as expected. Economically, higher income from the sale of milk (ISM) and the total income of dairy activity (TIDF) were seen, which resulted in higher gross margin (GM) for the high production system. High cluster had 22% higher annual income (sale of milk and animals) than the medium-high cluster. At the same time, the operating cost for production increased in smaller proportions (10%) determining higher gross margin (MB) (43%) and increased profitability (15%) compared to the medium cluster. Actual operating costs (AOC), between the two production systems showed that the higher cost was due to the animal feed (concentrate and roughage), with 75% of operating cost, for both

high and medium-high clusters and the second largest cost was due to the payment of labor, with an average of 12% of the operating cost. Considering the three scenarios, the average economic values of the characteristics that affect the profit of the producer for both clusters were in order: FAT: US \$ 3,776.07; PROT: US \$ 1,888.03; MP US \$ 1,258.69, LP: US \$ 1,258.69; Weight: US \$ 124.03; MORT: US \$ -14.30; INTAKE: US \$ -614.01; CI: US \$ -1,023.62; AFC: US \$ -2,724.29; SCC: US \$ -3,146.87. The decrease in the diet costs caused an increase in the profit percentage. On the other hand, the increase (+25 and +50%) in diet costs, would cause a decrease in farm profits (up to 25% in AFC with 50% increase in diet costs).

Conclusions: The economic values calculated for the characteristics related to volume of milk, fertility, milk quality, disease resistance, feed intake and mortality used in breeding programs resulted in increased profitability for milk production traits and weight for any scenario (I, II and III); increased profitability for milk quality characteristics for scenario II and III and decreased in profitability for fertility traits, consumption and mortality for scenarios I, II and III, and for characteristic related to the milk quality in scenarios II and III.

Genetics and Breeding

P04-004-038

Non-genetic factors affecting somatic cell count, milk urea content, test-day milk yield and milk protein percent in dairy cattle

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Objectives: This study had two aims. Firstly, to determine the associations of month of recording (MOR) and days in milk (DIM) with somatic cell count (SCC), milk urea concentration (MU) and milk production traits (i.e. test-day milk yield, TDM; milk protein percentage, PP and milk protein yield, PY). Secondly, to evaluate the associations among SCC, MU and milk production traits by analyzing individual monthly test-day records for dairy cows raised in the Czech part of the Czech Republic.

Materials and Methods: A total number of 33,881 individual test-day milk records involving 15,565 dairy cows for a 12-month period enrolled in Database Computer Center belonging to Czech Moravian Breeders' Corporation, Inc., Hradistko, the Czech Republic was used. Individual test-day milk samples were analyzed in the Milk Testing Laboratory of Buřtehrad. Somatic cell count was estimated using Bentley Instruments (Somacount 3000, the instrument utilize a laser based flow cytometry). Protein percentage was estimated also using Bentley Instruments (Bentley 2000, infrared transmission photometer). The direct specific enzymatic method UREAQVANT (made by Agroslužby Morava a Slezko) was used for the routine determination of the MU. Data analysis was performed using the Statistical Analysis System (SAS/STAT 9.1 User's Guide, 2004). To control the repeated measures on different test-day record at the same cow level, repeated measures statements were used in the statistical analyses. In order to allow the inclusion of repeated measures statements in the statistical model, a minimum of two dependent variables per statistical model was imposed for the statistical analysis by SAS (2004). Descriptive statistics (i.e. mean, SD, CV %, minimum, and maximum) were also computed for TDM, PP, PY, SCC, SCS, MU and DIM using SAS (SAS, 2004). Summary of the descriptive statistics of test-day records.

Results: Analysis of variance shows that MOR and DIM has highly significant ($P < 0.01$ or 0.001) effects on all studied traits. The coefficient of determination (R^2) varied from 4% (for SCC) to 84% (for PY). In general, the magnitude of the effect of these factors including in the models in SCC, SCS and MUN was small. Only 4.0, 7.0 and 18.0% of the variation in SCC, SCS and MU values, respectively were explained by the factors studied. It is clear that there are other sources that account for most of the variation in SCC and MUN (including nutritional factors) which were not included in this study. Coefficient of variation (CV %) varied from 8.92% (for PP) to 183.8% (for SCC). The R^2 varied from 41% to 69% for the studied traits, with SCS having the lowest R^2 coefficients in all three lactations. Very high standard deviation for SCC was reported also by Ng-Kwai-Hang et al (1985) who found that SCC in terms of thousands per milliliter of milk was the most variable component with an overall mean of 297 and a standard deviation of more than twice this. The R^2 for the GLM procedure that examined the relationship between the variables and MU was 0.47 using a large database ($n = 1,279,600$). The differences in the values of R^2 and CV% may be due to the different statistical analysis, breed, number of observations and/or fixed effects included in the statistical model. A negative linear relationship between milk yield and PP in the same milking was observed. TDM for PP less than 3.2% was 24.6 kg, meanwhile, it was 18.6 kg for PP > 3.8 .

Conclusions: Highly significant effects ($P < 0.001$) on SCC, MU and milk production traits were found for MOR and DIM. Therefore, the effects of environmental variables must be taken into consideration when cows are evaluated and when developing and comparing models to be used in adjusting data to provide the best estimates of genetic parameters for selection purposes in the dairy cattle evaluation. The results demonstrate the importance of surroundings and management practice. Therefore, management play a great role as source of variability and has a effective role in maintaining optimum SCC and MU levels for health status and nutrition.

Genetics and Breeding

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Identification Of Snps In Fcgrt Gene And Their Association With Gamma Immunoglobulin Concentrations In Buffalo (*Bubalus bubalis*)

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Objectives: Calf mortality causes a major economic loss to dairy production. In ruminants, a calf is born agammaglobulinemic because transfer of antibodies through syndesmochorial placenta does not occur. The neonate is completely dependent on the absorption of maternal antibodies from colostrum as a source of immunity against diseases which occurs non-selectively by pinocytosis facilitated by certain receptors of small intestine known as neonatal Fc receptors (FcRn). This is encoded by B2M and FCGRT gene. We attempted to detect SNP's in FCGRT gene in buffalo and ascertain their association with IgG concentration in dams' colostrum and calves' serum.

Materials and Methods: Indirect ELISA was performed to determine the Immunoglobulin-G (IgG) concentration in serum samples of eighty murrah buffalo calves born during August 2013 to February 2015 and colostrum of their respective dams. The dams and calves were separately ranked in ascending order of colostrum and serum IgG concentration, respectively. Animals (dams or calves) which fell in bottom one quarter and best one

quarter were subjected to DNA isolation and subsequent experimentation. Nine pairs of primers (three pairs for the last exon) were designed to amplify nine fragments corresponding to seven exons of FCGRT gene in buffalo. SNPs were identified by PCR-SSCP of nine fragments. The odds ratio was calculated to ascertain the SNPs that were significantly associated with IgG concentration either in colostrum or serum.

Results: The mean value of IgG in low and high yielder dams was 32.25 ± 1.76 and 70.33 ± 5.28 respectively. Similarly, the mean value of serum IgG in low and high yielder calves was 2.85 ± 0.34 and 16.51 ± 0.47 , respectively. The PCR SSCP analysis and sequencing of fragment 2 (342 bp) revealed three G288C, C302T and C122G SNPs. The PCR SSCP analysis and sequencing of fragment 3 (372 bp) revealed three alleles A, B and C that differed at nucleotide positions 40 and 332. Analysis and sequencing of fragment 4 (361 bp) revealed three patterns A, B and C and two SNPs viz. A75G transition in exon 4 and G314T transversion in intron 4. Polymorphism at 75th position (A to G) lead to sense mutation so change of amino acid from isoleucine (I) to valine (V). The PCR SSCP analysis and sequencing of fragment 7A (339 bp) revealed two alleles A and B that differed at nucleotide positions 139 and 270. The PCR SSCP analysis and sequencing of fragment 1 (308 bp), fragment 5 (392 bp), fragment 6 (250 bp), fragment 7B (330 bp) and fragment 7C (157 bp) did not reveal any SNP hence these fragments were observed as monomorphic. In calves, for the SNP G40T in fragment 3, the presence of G and T allele at 40th position was significantly ($P \leq 0.05$) differing in low IgG and high IgG groups. In dams, both the SNPs (A75G and G314T) in fragment 4, were found to differ significantly ($P \leq 0.05$) in low IgG and high IgG groups. In dams, in polymorphic fragment 4, the haplotypes (AG, AT and GT) were found to differ significantly ($P \leq 0.05$) in low IgG and high IgG groups.

Conclusions: We identified 9 SNPs, located in different exonic and intronic regions of FCGRT gene in murrah breed of buffaloes. Seven SNPs were located in intronic (non coding) regions while 2 SNPs corresponded to exonic (coding) regions, one being nonsynonymous, causing alteration of Isoleucine (Ile) to valine (Val) while the other being synonymous mutation. Both the SNPs (A75G and G314T) present in fragment 4 in dams exhibited a significant bearing on colostrum IgG. Similarly the SNP (G40T) present in fragment 3 in calves exhibited a significant bearing on serum IgG.

Genetics and Breeding

P04-004-040

Influence Of Mirna On Proliferation And Differentiation Of Bovine Satellite Cells On Different Stages Of In Vitro Myogenesis

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Objectives: The goal was to identify the role of miRNAs in skeletal muscle development in cattle breeds of different performance in order to understand the molecular mechanisms of myogenesis influencing beef gains and quality.

Materials and Methods: Samples of semitendinosus muscle were collected from 15 months-old bulls of beef (Hereford, HER & Limousine, LIM) and dairy (Holstein-Friesian, HF) breeds. Muscle samples were cleaned of other tissues, sliced and suspended in sterile

FBS with 10% addition of DMSO and then gradually cooled down to -80°C. Satellite cells were isolated according to the modified protocol of Baquero-Perez et al. (2012). Subsequently, cells were cultured in proliferation medium (10%FBS/DMEM/antibiotics). After reaching 80% of confluence cells were collected for further analysis (control condition) or proliferation medium was replaced by differentiation medium (2%HS/DMEM/antibiotics). After the 2nd and 6th day of differentiation cells were scraped and stored at -80°C until further analysis. Microarray analysis was performed using Bovine Custom miRNA Microarray 8x60K AMADID, (Agilent Technologies, USA). The total RNA from proliferating and differentiating cell samples was isolated using miRNeasy Mini Kit (Qiagen, USA) labelled and hybridized to microarray slides. The microarray output text files were analyzed using GeneSpring 13 (Agilent Technologies, USA). ANOVA with $p \leq 0.05$, FDR ≤ 0.05 and fold-change of at least 2.0 were used as the criteria of significance. To validate microarray data, Real-time RT-PCR analyses were performed. For this purpose miRCURY LNA First-strand cDNA Kit, miRCURY LNA SYBR Green master mix and miRNA primers (Exiqon, USA) were used. Statistical analyses were done using GenEx 6 (MultiD, Sweden). Ontological analysis was performed using PathwayStudio 11 (Elsevier, Holland).

Results: The analysis of miRNA expression was performed in bovine satellite cells (BSC) at early and late stages of differentiation (2nd and 6th day) as well as in proliferating cells (80% confluence) used as a control. Microarrays were performed separately for each breed (HF, HER, and LIM). For Limousin, 44 miRNAs showed differential expression in differentiating (day 2 and 6) vs. proliferating cells. Similar analysis for the BSC derived from Hereford bulls allowed identification of 34 differentially expressed miRNAs. In the case of Holstein-Friesian bulls 39 miRNAs were identified. Global analysis of the miRNA expression in the aforementioned conditions showed a group of 13 miRNAs manifesting similar expression trends on 2nd and 6th day of differentiation in all examined breeds. Ontological analysis of identified miRNAs has revealed that they take part in the following biological processes: myoblast proliferation, myoblast differentiation, satellite cell activation and myogenesis. Additionally, the three miRNAs, whose expression was similar for beef but different from dairy bulls, were identified in both analyzed conditions (2nd day of differentiation vs. proliferation and 6th day of differentiation vs. proliferation). The cluster analysis showed that the expression of miRNAs in BSC from beef bulls shows a greater similarity between 2nd and 6th day of differentiation while expression of miRNAs in BSC of dairy breed has a greater similarity between the proliferating cells and cells entering differentiation (2nd day).

Conclusions: It seems that the identified group of 13 miRNAs could be classified as common regulators of myogenesis in cattle, since they change their expression during various steps of differentiation in a similar manner in all examined breeds. The identified 3 miRNAs (miR-133b, miR-206 and miR-2487) specific for HER & LIM may be the main regulators of myogenesis in beef breeds responsible for rapid and more intensive growth of myotubes observed *in vitro* and the enhanced muscle mass gains *in vivo*. Furthermore, cluster analysis has shown possible higher miRNA-related differentiation potential of BSC isolated from muscle tissue of beef breed bulls.

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Genetics and Breeding

P04-004-041

Genome-wide association study of immunoglobulin absorption in neonatal Holstein calves

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Objectives: Absorption of colostral immunoglobulins in neonatal calves is critical to health early in life, but appears to be vary widely between calves. The objective of this study was to identify single nucleotide polymorphisms (SNPs) associated with the efficiency of absorption of colostral immunoglobulins in neonatal Holstein calves.

Materials and Methods: 102 Holstein heifer calves born without assistance on 3 farms were enrolled in the study. Blood samples were collected between 24 and 36 hours of age. Whole blood was stored for genetic analysis, and serum was stored for IgG quantification. Samples of colostrum fed to each calf were also stored for IgG quantification. IgG concentration in the colostrum and serum samples was measured by radial immunodiffusion assay, and apparent efficiency of absorption (AEA) was calculated for each calf. A genome-wide association study was performed using the Illumina Bovine HD array (over 777,000 SNPs) to identify SNPs associated with AEA in which AEA was treated as a quantitative trait.

Results: AEA ranged from 7.7% to 59.9%. The mean AEA in the calves was 28.1%. After correction for population stratification using Efficient Mixed-Model Associated Expedited (EMMAX), SNPs on chromosome 2 ($p_{\text{raw}} = 1 \times 10^{-7}$) and chromosome 13 ($p_{\text{raw}} = 1.3 \times 10^{-6}$) were found to be associated with improved efficiency of absorption of IgG. These SNPs associated with improved AEA were found in 4 and 5 calves, respectively. Multiple candidate genes were found in these areas. No SNPs were associated with a decreased AEA.

Conclusions: There is wide variation in the efficiency of IgG absorption in Holstein calves. Further characterization of sequence variation in these two regions may provide an economical means to select for cattle with improved efficiency of absorption, allowing for reduced rates of failure of transfer of passive immunity.

Genetics and Breeding

P04-004-042

Analysis of the genetic population structure of the Crioula Lageana cattle breed

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Objectives: Knowledge of the population structure of Crioula Lageana breed is essential to provide the foundation for understanding the evolution of genetic parameters of this population, assisting in improving conservation programs and sustainable use this genetic resource. Thus this study aims to characterize the population structure of locally adapted Crioula Lageana Brazilian cattle breed to assess genetic diversity.

Materials and Methods: Data from 1,638 animals from Santa Catarina Plateau from the database of the Brazilian Association of Crioula Lageana Cattle Breeders (ABCCL), collected over a 38 year period were used. Information included the registration number, date of birth, sex, dam and sire registration number, and farm. Data analysis of the genetic structure of bovine breed population Crioula Lageana was performed using the program ENDOG v.4.6, and the following parameters were calculated: pedigree integrity index (PI), inbreeding coefficient (F), inbreeding rate (Δf), effective population size (Ne), average relationship coefficient (AR), effective founders (fe), effective ancestors (fa), genetic conservation index (GCI), generation interval (GI) and Wright's F statistics.

Results: Pedigree information is in the fifth generation, and only 44.87% of the animals had a known sire, while 40.42% have a known dam. Effective population size (Ne) ranged from 72.53 in complete generations to 143.90 in maximum generations, which is above the minimum value considered by the Food and Agriculture Organization of the United Nations to guarantee maintenance of maximum genetic variability. Inbreeding (F) and Average Relatedness (AR) were 0.34% and 0.91%, respectively. The effective number of founders (fe) and ancestors (fa) in this study were 29 and 28 animals, respectively, and only ten ancestors were responsible for 50% of the genetic variability of the whole population. The average generation interval was 5.84 years in the paternal line and 7.70 in the maternal line. Wright's F statistics indicate low genetic distances between subsets in relation to the total population ($F_{st}=0.0015$), between individuals with respect to their subpopulation ($F_{is}=-0.0027$) and between individuals in relation to the total population ($F_{it}=-0.0012$). This indicates a low genetic differentiation in the population studied. Analysis of population genetic structure of Crioula Lageana cattle indicates that, despite the small number of animals with known parentage and considerable loss of genetic variability by the constant use of a few sires, the population showed good genetic management, low inbreeding, low genetic differentiation among subpopulations and adequate effective population size for breed preservation.

Conclusions: The analysis of the genetic structure of the Crioula Lageana cattle breed population indicates a reduced number of known animal pedigree and considerable loss of genetic variability for the development of a population with a narrow genetic base. The population has low levels of inbreeding, low genetic differentiation among subpopulations and an effective population size above the recommended by FAO to maintain maximum genetic variability over the generations.

Genetics and Breeding

P04-004-043

Population viability analysis of the Crioula Lageano cattle

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Objectives: The objective of this study was to perform a population viability analysis with the locally adapted Crioula Lageana Brazilian cattle breed of cattle to study its demographic and genetic parameters, as well as verify the interference of factors that may affect the survival and development this population over a 500-year timespan.

Materials and Methods: The interaction between demographic, environmental and genetic factors on the population of Crioula Lageana animals was evaluated using the VORTEX software. There were 128 simulated hypothetical scenarios. The input variables in the program were as follows: initial population consisting of 1,408 animals, with 205 males and 1,203 females (current population); 100 repetitions performed in each scenario; 100 and 500 years simulation time; land capacity (K) of 500 animals; no removal of animals and removal of 100 animals from the population annually; sexual maturity at 2 years of age and maximum reproductive age of 15 years; one offspring per pregnancy with 50% of likelihood of being born male and female; 5:95; 15:85; 30:70 and 50:50 ratio of males to females in a population of 100 animals intended for breeding annually; inbreeding depression with a lethal amount of 3.14 equivalents per diploid genome; 5, 10, 25 and 40% of mortality at the first year of life and 2.5% over one year of age as well as five catastrophic events one every 20 years for the scenarios with up to 100 years, and a catastrophic event every 100 years for the scenarios up to 500 years. Using demographic, genetic and stochastic information of the Crioula Lageana breed the expected heterozygosity (He) and inbreeding coefficient (F), the deterministic growth rate (det.r), the population size, the probability of extinction as well as the population dynamics parameters were calculated.

Results: The possibility of extinction of Crioula Lageana breed over the next 500 years was remote, even in less favorable scenarios. The deterministic growth rate (det.r) was always positive during the evaluated period, and the mortality of animals up to one year of age was the most important factor that affected this parameter. The population size increased rapidly until the carrying capacity (k) was reached, which was a limiting factor for population growth. The level of retained heterozygosity (He) in the population in the worst scenario was above 80% and the coefficient of inbreeding (F) was always below 0.20 at the end of the 500 years of simulation.

Conclusions: The assessment of population viability of Crioula Lageana the Santa Catarina Plateau breed showed that this population has no apparent long-term risk of extinction as a result of its high genetic diversity and adequate demographic structure, and the area's carrying capacity is the main limiting factor for population growth. Farmers should adopt measures to ensure a low mortality rate in the first year, and a high male:female ratio.

Genetics and Breeding

P04-004-044

Determination Of Haplotypes In Beta-2 Microglobulin Gene And Their Effect On Immunoglobulin-G Levels In Buffaloes

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Objectives: Neonatal calf mortality is one of the major causes of economic losses in livestock production particularly in buffaloes. The new-born calves carry no antibodies in their blood stream at birth due to the uterine barrier. Therefore their only source of gaining immunity is through passive transfer of immunoglobulins. The passive transfer of immunity is determined by the neonatal receptor i.e. FcRn, which is a heterodimer composed of two subunits viz. FCGRT and Beta 2

microglobulin. We resorted to explore polymorphism/haplotypes in β_2 -microglobulin (β_2M) and ascertain their effect on IgG levels in dams' colostrum and calves' serum.

Materials and Methods: The β_2M gene in buffaloes was amplified in forty newly parturated murreh buffaloes and their respective new born calves by employing seven pairs of primers for amplification of both exonic and intronic regions i.e., fragment I (206 bp: full exon 1), fragment II (296 bp: partial intron 1), fragment III (287 bp: full exon 2), fragment IV (227 bp: partial intron 2 and full exon 3), fragment V (226 bp: partial intron 3), fragment VI (201 bp: initial partial exon 4) and fragment VII (405 bp: remaining partial exon 4) in the DNA samples of Murreh buffaloes which were designed on the basis of sequences of cattle (*Bos taurus*) in public database at NCBI (AC_000167.1). Genomic DNA was extracted from 5 ml of venous blood. The quality, purity and concentration of DNA was checked. The reaction mixture and PCR programme were optimized to achieve the satisfactory level of amplification for all the seven fragments. Polymerase chain reaction- single stranded conformational polymorphism (PCR-SSCP) technique was used to explore the polymorphism in β_2M gene of Murreh buffaloes. The different banding patterns were identified and each representative pattern was sequenced. The haplotypes in dams and calves were determined. Indirect ELISA was done to estimate IgG concentrations in colostrum (first milking) whey and serum of new born calves (6 to 12 hrs post birth). The effect of different haplotypes was evaluated on IgG concentrations by using PROC GLM module of SAS9.3 software.

Results: The PCR-SSCP analysis and sequencing of different patterns of fragment I revealed two genotypes AA and BB with only one SNP viz. C45G in 5'UTR. The analysis and sequencing of fragment II revealed two genotypes AA and BB due to SNPs viz. T22A, T23A and G256A in intron 1. The analysis and sequencing of different patterns of fragment III revealed only one SNP viz. T204C in exon 2. The genotypic frequencies of AA and BB genotypes were 95 and 5 % respectively. Polymorphism at 204th position (T to C) did not lead to alteration of amino acid hence was a synonymous (silent) mutation. The PCR-SSCP analysis and sequencing of PCR products of fragment IV, V and VI did not revealed any SNP hence, exon 3, intron 3 and exon 4 of β_2M gene were observed as monomorphic. The PCR-SSCP analysis and sequencing of different patterns of fragment VII revealed four SNPs viz. G80A, A101C, A208G and A239G in fourth exonic region (3' UTR). The patterns were designated as AA, BB, CC and DD genotypes whose overall genotypic frequencies were 47.5, 30, 15 and 7.5 % respectively. The identified 9 SNPs resulted in 7 different haplotypes in buffalo dams and 14 different haplotypes in calves. The mean IgG concentration in dams' colostrum and calves' serum were 51.71 ± 5.99 and 11.68 ± 0.75 mg/ml respectively. The effect of different haplotypes was evaluated on IgG concentrations. The least square analysis of variance revealed a non-significant effect of dam haplotype on colostrum IgG concentrations as well as calf haplotype on serum IgG concentrations.

Conclusions: Nine SNPs were identified in seven amplified intronic and exonic regions of the β_2M gene. Out of these SNPs eight SNPs lied in non coding region while one SNP lied in coding region (T204C in exon 2). This nucleotide change was synonymous type i.e., no change in amino acid occurred due to this SNP. These SNPs resulted in 7 haplotypes in dams and 14 haplotypes in calves. ANOVA did not reveal any significant effect of haplotype on IgG concentrations (either dam or calf).

Evaluation of morphometric data of Criollo Lageano cattle

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Objectives: The objective of this study was to evaluate the body measurements of the cattle breed Creole Lageana at different ages in the three regions of Santa Catarina plateau, in order to establish the patterns of variation of these measures, contributing to the phenotypic characterization of the breed.

Materials and Methods: Body measurements were collected from 346 animals from three different regions (Coxilha Rica, Campos de Caçador and Campos de Curitiba) of the Plateau of Santa Catarina State. The following measurements were taken: body length, height at withers, thoracic girth, rump height, rump width and rump length. The morphometric measurements were evaluated in terms of age, gender and region of origin. The statistics analysis included an analyses of variance, correlation, principal factors, as well as cluster, discriminant and canonical analyses.

Results: All measurements, except for rump height, showed a statistical difference between males and females, demonstrating the sexual dimorphism present in this breed. In relation to age, it was observed that rump height varied until animals between 3 and 5 years of age; while rump width and thoracic girth varied until animals between 7 and 9 years. Height at withers and rump length stabilized in animals between 9 and 11 years, and the last trait to stabilize was body length, which only stop growing in animals above 11 years of age. The cluster analysis, based on morphometric information of this breed, showed a small distance between animals from the region of Campos de Curitiba when compared to animals from the regions of Coxilha Rica and Campos de Caçador. The discriminant analysis identified that an increase in body length, height at withers and rump length were associated with a decrease in rump height and rump width. The linear measurements that presented the greatest correlation were thoracic girth, rump width and rump length, with values above 0.80.

Conclusions: The analysis of body measurements indicated that the Criollo Lageano cattle breed show sexual dimorphism, low differentiation between regions in the Plateau of Santa Catarina State and late finishing, suggesting that farmers do not select the animals for early termination, but that they should join forces for the inclusion of this breed in added value production systems such as certification of Protected Designation of Origin of its meat.

Genetics and Breeding

P04-004-047

Performance and carcass traits in locally adapted Brazilian cattle

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Objectives: The objective of the present study was to compare quantitative and qualitative carcass characteristics of Pantaneiro, Curraleiro Pe Duro and Nelore Brazilian cattle breeds under commercial feedlot conditions.

Materials and Methods: Fifteen males, approximately two years of age, of Pantaneiro, Curraleiro Pe-duro and Nelore breeds were evaluated after 17 days of adaptation for 97 days. The animals were reared as one group with the same environmental and treatment conditions and fed with 70% roughage, 30% concentrate, mineral salt and water ad libitum. Animals were weighed after 14 hours of fasting at the beginning and the end of the experimental period. The eye muscle area and fat thickness on the eye muscle area were determined in the cross section of the 12th and 13th ribs from the left half carcass in Longissimus lumborum, using ultrasound (Aloka SSD-500) at the beginning and end of the experimental period. Animals were slaughtered on three dates, with one third of each genetic group per day and the conformation (CF) and physiological maturity (FM) of the carcasses were evaluated. Carcass length (CL), leg length (LL), arm length (AL), thigh thickness (TT), and arm circumference (AC) were recorded using a measuring tape. Sirloin was analyzed for meat color using a colorimeter (Konica Minolta®) to evaluate the brightness (L^* 0 = black, 100 = white), the intensity of the red color (a^*) and the intensity of the yellow color (b^*). The statistical analysis included an analyses of variance, correlations, principal factors, as well as discriminant and canonical analyses.

Results: Nelore animals had the highest initial and final weight, physiological maturity and leg length (346.8 kg; 475.46 kg; 14.11; 74.54 cm, respectively). The initial weight of the animals had a significant effect on conformation, carcass length, arm circumference and thigh thickness, however arm length showed no effect. For the qualitative carcass characteristics of sirloin, the Nelore breed presented more brightness and intensity of red color compared to the other breeds, however, the yellow color was not different between breeds.

Conclusions: The Nelore Brazilian cattle breed presented the best carcass quantitative and qualitative characteristics when compared with Pantaneiro, Curraleiro Pé Duro breeds, under commercial feedlot conditions.

Herd Health Management: Beef

P01-001-017

Prevalence and risk factors of injection site lesions in UK beef cattle

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Objectives: Injectable veterinary medicines are widely used in cattle, and in particular vaccines are used on large numbers of animals in the herd. The formation of injection site lesions (ISLs) is a risk when using injectable products, and has potential consequences for meat quality, animal welfare and beef industry revenue.

Materials and Methods: This study collected carcass observation data in abattoirs to determine ISL prevalence in UK beef animals, which was linked with a questionnaire investigating cattle vaccination techniques amongst UK beef farmers.

Carcasses were inspected visually by Food Standards Agency and slaughterhouse staff at both control points and any lesions or abnormal tissue was removed from the carcass. The tissue was labelled with the date and kill number. Where possible a gross morphological description was recorded.

A questionnaire was developed and distributed in paper format and online to cattle farmers in the UK between September and November 2011 using a convenience sample. The questionnaire contained 23 questions about cattle vaccine uptake, and collected data on how the vaccines were stored and administered.

Results: There was a trend (p=0.22) towards a difference in distribution between site of vaccine administration (60% in neck, 33% in rump) and site of lesions observed at the abattoir (42% in neck, 47% in rump)

When asked 'When administering this vaccine by injection (using e.g. a syringe, vaccinator gun), which of the following apply on your farm?' 43% of respondents started each vaccination session with a new needle or changed needles when they became broken or blunt.

The ISL prevalence was 4.1%, and the difference between sites being used for vaccination and the distribution of ISLs on carcasses suggested that factors other than vaccination were contributing to ISL incidence. Questionnaire responses highlighted deficits in compliance such as site of injection and correct needle usage.

Conclusions: The role of the veterinary surgeon in knowledge transfer is crucial in providing practical injection advice when prescribing vaccines and other veterinary medicines. This presentation will highlight practical factors that can be addressed by veterinary surgeons to reduce ISL formation in beef animals.

Herd Health Management: Beef

P01-001-019

On-farm mortality and related risk factors for Estonian beef cattle

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Objectives: On-farm cattle mortality (euthanasia and termination) is involuntary loss of an animal associated with economic consequences to the farmer. High on-farm mortality is one of several indicators of suboptimal animal health and welfare. So far, scarce information about mortality and related risk factors exist in beef cattle population. Objective of this study was to assess beef cattle mortality in Estonian beef herds and discover potential risk factors associated with mortality.

Materials and Methods: Animal level data of all cattle in Estonian beef herds (consisting of more than 75% of beef cattle) was extracted from the Estonian Agricultural Registers and Information Board database for the years 2013 and 2014. Study population consisted of 58,708 cattle from 1,186 herds and 69,413 animals from 1,288 herds for years 2013 and 2014, respectively. The associations between possible risk factors (age, breed, gender, herd size, region) and mortality was analysed with Weibull proportional hazards models with farm as random effect. Interaction terms were introduced as separate variables in the model. Statistical analyses were performed with Stata MP version 14 (StataCorp LP, College Station, USA).

Results: Among 48,918 animal-years 1,166 animals died or were euthanized with resultant mortality risk of 1.99% and mortality rate of 2.38 per 100 animal-years in 2013. In 2014 1,030 deaths and euthanasias occurred per 57,602 animal-years resulting in a mortality risk of 1.48% and 1.79 per 100 animal-years. Significant decline in cattle mortality within two years could be identified (p < 0.001). According to multivariable Weibull proportional hazard random effect model the mortality risk was highest among male cattle up to one years old (HR = 1.20, 95% CI 1.05; 1.37) followed with females in the same age group (the baseline group). The hazard of dying declined with increasing age. In general, male cattle from older age groups (>12 months) experienced lower risk of mortality compared to females. An increased mortality of cattle from 'other' breed group consisting animals from breeds underrepresented in the Estonian beef herd's was established (HR = 1.37, 95% CI 1.13; 1.66, p = 0.002) compared to Aberdeen Angus, which was the baseline group in the model. The effect of herd size was dependent on the region. Mortality risk was highest among animals in largest herds (≥75 cows) from South of Estonia (HR = 2.11, 95% CI 1.52; 2.95, p < 0.001) followed with those kept in herds with 50-75 cattle in the same region (HR = 1.94, 95% CI 1.38; 2.73, p < 0.001) compared with baseline group (smallest herds with <10 cows in South of Estonia).

Conclusions: Results of our study demonstrate on-farm mortality rate comparable with other studies and declining between years 2013 and 2014. This study revealed several factors influencing beef cattle mortality in Estonia. More research is needed in order to advise beef cattle farmers in their decisions when reducing on-farm mortality.

Herd Health Management: Beef

P01-001-020

Evaluation of on-arrival vaccination and deworming on stocker cattle health and growth performance

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Objectives: The effect of vaccinating beef calves arriving to a pasture-based growing (stocker calf) system is poorly documented and potentially detrimental because the immune system of stressed calves

may not be able to efficiently process additional antigens. In the short-term, deworming cattle may not overcome the detrimental effects of parasitism at arrival. Our objective was to evaluate the effect of on-arrival vaccination and deworming on bovine respiratory disease (BRD) incidence, mortality, and growth of stocker calves in the southeastern United States.

Materials and Methods: Calves (n=80) received from an order-buyer through typical auction market systems were stratified by d-3 weight and fecal egg count into 20 pens of 4 animals each. Pens were randomly assigned to treatments in a 2x2 factorial design to test vaccination at arrival (d0 modified-live BHV-1/PI3V/BVDV1/BVDV2/BRSV and clostridial vaccine or not) and deworming (d0 oral fenbendazole and levamisole or not). Body weight and blood was collected days 0, 14, 28, 42, 56, 70 and 85. Fecal egg counts were measured days -3, 28, 56, and 85. Clinical signs of BRD (depression, anorexia, rapid respiratory rate, cough, nasal discharge, and rectal temperature of ≥ 40 C) were monitored daily. Treatment effects on BRD incidence, mortality, and growth were tested using Poisson, logistic, or linear regression, respectively ($\alpha \leq 0.05$).

Results: BRD incidence was greater for calves with d0 vaccination (RR=3.2), high (≥ 40 C) fever at day 0 (RR=6) and higher d-3 FEC (RR=1.2 per 100 epg). Mortality was greater for d0 vaccination (OR=8.3) and high fever (OR=41.6). Growth was lower for d0 vaccination (-4.7 kg), moderate (39.4-39.9 C) and high fever (-10.9 kg and -7.3 kg, respectively), and number of times treated for BRD (-7.9 kg/treatment). Deworming at arrival was not found to be significantly associated with BRD morbidity, mortality, or weight gain.

Conclusions: Health and growth performance of stocker calves may sometimes be adversely affected by vaccination at arrival. Deworming stocker calves at arrival may not always be beneficial to health or performance in the first 85 days after arrival. Degree of parasitism, as evidenced by fecal egg count, and presence of fever at arrival may be important predictors of subsequent BRD diagnosis. Both of these factors may also be important confounding variables to control for during design or analysis of BRD studies.

Comments: A contribution of the Beef Cattle Population Health Program at Mississippi State University. Supported by the Mikell and Mary Cheek Hall Davis Endowment for Beef Cattle Health and Reproduction.

Herd Health Management: Beef

P01-001-021

The participation of the bovine respiratory disease complex on the mortality and morbidity rates in a feedlot from southeastern Brazil

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Objectives: This study investigated the contribution of bovine respiratory disease (BRD) towards the development of morbidity and mortality in a beef cattle feedlot located in southeastern Brazil during a two-year-period (2013-2014).

Materials and Methods: All steers with clinical manifestation of disease were immediately separated from the particular pen and examined by the on-site veterinarian. Data relative to clinical diagnoses, therapy, morbidity, and mortality were tabulated daily into a Microsoft Excel spreadsheet. This information was used as input data for the results of this study. The evaluation used for the characterization of respiratory disease was based on the DART system (Zoetis, Florham Park, NJ) with modifications from Wilson et al. (2015). Clinical diagnoses were classified into seven large predetermined groups: BRD, feed refusal, clostridial diseases, lameness, bloat, trauma, and polioencephalomalacia (PEM). An estimation of the economic impacts associated with BRD-related morbidity and mortality was also determined.

Results: During this period, 188,862 steers were on feed. The morbidity rate was 7.05% (13,315/188,862), with mortality at 0.64% (1,214/188,862). The principal causes of morbidity were BRD (6.13%; 11,577/188,862), lameness (0.29%; 550/188,862), trauma (0.21%; 406/188,862), clostridiosis (0.13%; 242/188,862) and polioencephalomalacia, PEM (0.12%; 222/188,862). Predominant causes of mortality were BRD (0.21%; 397/188,862), trauma (0.17%; 329/188,862), clostridiosis (0.13%; 242/188,862), other diseases (0.08/157/188,862) and PEM (0.02%; 46/188,862), resulting in 32.7% (394/1,214), 27.1% (329/1,214), 19.9% (242/1,214), 12.9% (157/1,214) and 3.79% (46/1,214) respectively, of the animals that died during this period. When all sick cattle were considered, BRD (86.9%; 11,577/13,315) was the principal cause of morbidity, followed by lameness 4.13% (550/13,315), trauma 3.05% (406/13,315), and clostridiosis 1.82% (242/13,315). BRD-associated mortality was estimated at \$14,334.00/10,000 cattle; morbidity due to BRD resulted in \$16,315.40/10,000 cattle. It was projected that the economic effects due to BRD-associated morbidity in Brazil were \$6.31 million/annum, while losses due to mortality were \$5.54 million, resulting in an annual loss of \$11.85 million.

Conclusions: This is the first study that investigated the incidence of BRD in feedlot cattle from Brazil, and the results herein described indicate that BRD contributed significantly to the development of mortality and morbidity of cattle on feed.

Herd Health Management: Beef

P01-001-022

Evolution of seroprevalence for BHV-1 gE in a French herd vaccinated with a live bovine herpesvirus type 1 marker vaccine.

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Objectives: The objective of the present field study was to determine the evolution of seroprevalence for Infectious Bovine Rhinotracheitis (IBR) due to Bovine Herpesvirus type 1 or BoHV-1 (BHV-1 gE) in a French herd vaccinated with a live bovine herpesvirus type 1 marker vaccine.

Materials and Methods: The herd vaccination programme started in November 2008 in a large beef herd located in Normandy with a high seroprevalence of IBR. 74 % of heifers aged from 14 to 30 months and vaccinated with a live marker vaccine (Bovilis® IBR Marker Live, MSD Santé Animale) had BoHV-1 gE antibodies before beginning the herd protocol. All cattle within this herd were vaccinated with the same live

marker vaccine. Calves were vaccinated intranasally at two weeks of age, revaccinated at three months and thereafter boosted every six months. Cattle aged three months or more were vaccinated once intramuscularly and revaccinated every six months. All the animals aged between 12 and 48 months were sampled every six months to evaluate their BoHV-1 gE antibody status. Biosecurity measures were also applied and every positive animal was removed.

Results: In this field study, a total of four hundred animals, including calves, were vaccinated with the live marker vaccine. Results showed that the incidence of new seroconversion (BHV-1 gE seroincidence) decreased over time (from 5 percent BHV-1 gE seroincidence in November 2009 to 0 percent BHV-1 seroincidence in November 2012). As a result, the herd BHV-1 gE seroprevalence decreased also over time (from 50 percent BHV-1 gE seroprevalence in May 2009 to 0 percent BHV-1 gE seroprevalence in May 2014).

Conclusions: It can be concluded that vaccination with a live IBR marker vaccine could significantly contribute, if associated to hygienic measures and to a specific herd management, to the eradication of BoHV1 in a large herd with a high seroprevalence under field conditions.

Herd Health Management: Dairy

P02-002-096

Mineral supplement responses of grazing cattle under tropical conditions in CubaGutiérrez Odilia¹, Gutiérrez B. Odilia² and Mineral group of Animal Science Institute¹Fisiología y Bioquímica, Instituto de Ciencia Animal, San José de las Lajas, Mayabeque, ²Fisiología y Bioquímica, Instituto de Ciencia Animal, San José de las Lajas, Mayabeque, Cuba

Objectives: In Cuba, the grazing cattle are developed in areas with different nutritional deficiencies such as nitrogen and mineral elements. The objective of these work was determine the principal mineral deficiencies in dairy grazing cows for to know the mineral supplement responses of grazing cattle under tropical conditions.

Materials and Methods: An interesting work was conducted during 15 years in West, Center and East regions where the principal mineral deficiencies in pastures were: nitrogen, calcium phosphorus, copper, zinc and manganese. Mineral premixes were supplemented to grazing cattle depending of productive and reproductive purpose and the principal deficiency founded in previous analysis of pastures. Dry matter, nitrogen and organic matter were determined as AOAC (2005), macro and micro minerals by atomically absorption and phosphorus for colorimetric method.

Results: Increase in milk production and composition such as a better alimentary efficiency were found when appropriate mineral premix was offered. Five milking enterprise from west areas presented acid soils with organic matter content above 3% and pastures middle values of nitrogen, phosphorus, copper and zinc of 11.2, 10.4, gkg⁻¹; 8 and 20 mgkg⁻¹ respectively. A mineral premix containing deficient minerals improves milk production in one kg/cow/day. Cows and heifers in Center Region presented low levels of blood copper and reproductive disorders which were improve with injectable copper. East Region presented soils with pH between 6-7 and pastures protein levels ≥ 8%. Blood indices of cows and heifers showed low levels of hemoglobin, hematocrit, phosphorus, copper and total proteins which were better when a new mineral premix was offered.

Conclusions: In conclusion, it was necessary a mineral premix (calcium, phosphorus, sodium magnesium and microelements) addition in grazing cattle under pastures of poor quality in order to increased productive and reproductive indicators.

Comments: Keywords: mineral premix, milk production, cattle.

Herd Health Management: Dairy

P02-002-097

An assessment of immune and stress responsiveness in Holstein-Friesian cows selected for high and low feed conversion efficiencyJosh Aleri¹, Brad C. Hine², Michael Pyman¹, Peter Mansell¹, Bill Wales³, Bonnie Mallard⁴, Andrew D. Fisher¹¹Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Werribee, ²Agriculture Flagship, CSIRO, Armidale, NSW, ³Agriculture Research and Development Division, Department of Economic Development, Jobs, Transport and resources, Ellinbank, Victoria, Australia, ⁴Department of Pathobiology, Ontario Veterinary College, University of Guelph, Ontario, Canada

Objectives: selecting animals for enhanced immune responsiveness and feed conversion efficiency are herd improvement strategies. Information on the relationship between immune responsiveness and feed conversion efficiency in dairy cattle is limited. Therefore, to provide insights into the potential benefits of selecting animals with enhanced general immune responsiveness the objective of this study was to assess the relationships between immune responsiveness, stress responsiveness and feed conversion efficiency of cows identified as extremely high and low feed conversion efficiency phenotypes.

Materials and Methods: The study utilized 16 Holstein-Friesian cows in their 3rd - 4th lactation, and identified as having either extremely high (n = 8) or extremely low (n = 8) feed conversion efficiency. The high feed conversion efficiency group had a mean of -0.825 ± 0.29 kg/DM/day residual feed intake and the low feed conversion efficiency group had a mean of 0.699 ± 0.19 kg/DM/day residual feed intake. Residual feed intake was defined as the difference between an animal's actual feed intake and its expected feed intake which was determined by regression of dry matter intake against main body weight and growth rate. A commercial vaccine was used to induce measurable antibody (AMIR) and cell-mediated (CMIR) adaptive immune responses and assess general immune responsiveness at days 0, 8 and 10 of the immunization protocol. AMIR were assessed using ELISA whereas CMIR were assessed using delayed type hypersensitivity reactions. Stress responsiveness was assessed by measuring changes in plasma cortisol concentrations in response to yarding and handling.

Results: Comparisons of AMIR measures revealed no significant differences between the high and low feed conversion efficiency phenotype groups on day 0, 8 or 10 (P=0.585, 0.978 and 0.343) respectively. Similarly, no difference was observed between feed conversion efficiency phenotype groups for CMIR measures (P=0.546). Pairwise comparisons revealed that AMIR measures were significantly higher on day 8 and 10 compared to day 0 (P=0.0001 and P=0.036, respectively). Differences between day 8 and 10 AMIR measures were not significant (P=0.975). Lower cortisol concentrations were observed in the high feed conversion efficiency phenotype cows as compared to their low feed conversion efficiency group on day 10 but only approached significance (P=0.079). No significant difference in cortisol levels were observed between the groups on day 8 (P=0.394) despite the low feed conversion efficiency group recording higher cortisol concentrations as compared to the high feed conversion efficiency group. Furthermore cortisol responses were significantly increased on day 10 relative to day 8 (mean difference = -2.80 nmol/L, 95% CI $(-5.11 - -0.49)$ nmol/L, P=0.021). A significant negative (but favourable) relationship was observed between AMIR and stress responsiveness, (R=-0.44, P=0.043) in the current study. In contrast, stress responsiveness and CMIR were not correlated (R=0.135, P=0.309).

Conclusions: To the author's knowledge, the relationship between immune responsiveness phenotype and feed conversion efficiency phenotype has not been investigated previously in dairy cattle. This study provides preliminary evidence that cows selected for feed conversion efficiency may have improved stress coping abilities and immune responsiveness. Results from this study support our hypothesis that enhanced immune responsiveness is correlated with reduced stress responsiveness, however validation of our findings in a larger population of animals is required.

Herd Health Management: Dairy

P02-002-098

Improving colostrum management in dairy calves by blood IgG measurement combined with recording and analyzing colostrum management data.

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Objectives: Neonatal Calf Diarrhea (NCD) is a common disease affecting newborn calves. To reduce the incidence of NCD it is important to use a preventive approach. Vaccinating the dam is a proven preventive method to increase specific 'protection' and good colostrum management is a crucial element for a newborn calf to get a good start. The aim of the study is to gain more insight in the correlation between colostrum management and blood IgG levels of newborn dairy calves.

Materials and Methods: The study was conducted in The Netherlands using data from a 13 dairy farms. A colostrum management form was used to record data during the first 24 hours after calving (animal identification, calving date, time of calving, time of 1st colostrum feeding, amount of colostrum feed, IgG concentration). Information from a total of 58 dairy calves was recorded in the form and used for analysis. Blood samples from these calves were obtained from 2nd and the 5th day after birth in order to determine blood levels of IgG.

Results: Sixty-two percent of the examined calves had a low blood IgG level (IgG < 15 g/L), the remaining 38% had a high blood IgG level (>15 g/L). Most calves with a low blood IgG level received 5 liters colostrum or less in the first 24 hours, which was significantly lower compared to the calves with a high blood IgG level. There were no differences between calves with high and low IgG blood level in the interval birth-first feeding (OR=1.2, p=0.78, 95% ci 0.3-4.9), the interval second-third feeding (OR=1.3, p=0.7, 95% ci 0.4-4.6) and the amount of colostrum fed in the second and third feeding (OR=1.1, p= 0.85, 95% ci 0.4-3.4), (OR=1.2, p=0.75, 95% ci 0.3-4.5).

Conclusions: Conclusion of this study is that most calves still don't receive enough colostrum. Most calves with a low blood IgG level received colostrum directly after birth, but a strong trend was seen that the amount of colostrum in the first feeding was not enough and the second feeding was offered too late. Not all farmers milked out the dam completely directly after birth, which raises concerns. This study emphasized that by recording and analyzing colostrum management data in combination with determining the blood IgG level of dairy calves, a customized advice can be given.

Herd Health Management: Dairy

P02-002-099

Automated measurement system of body temperature in calves using infrared thermography

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Objectives: Infectious diseases are a major concern in cattle industry.

Young calves are susceptible to infection of pathogens because of their immature immune system, and hence they often lead to a death with diarrhea or pneumonia after birth.

Early detection of sick animal is important to implement effective treatment and disease control. Many of sensing techniques of health condition in animals are invasive, and they usually require capture of animals, and collection of clinical samples. Infrared thermography is a non-invasive technique, and is useful for early detection of animals with infectious diseases by monitoring body temperatures continuously.

Materials and Methods: Young Holstein or Japanese Black calves (5 to 6 heads per group) managed and maintained by calf feeder in several farms were used in this study. Each group was recorded their clinical signs and amount of milk other than body temperature every day for one month. We tested whether our automated system can distinguish individuals by a visual camera, and can measure their facial, particularly ophthalmic max temperatures by using an infrared camera (InfRec TS9100, Nippon Avio) when calves are drinking milk fed by robots, in order to monitor their health condition continuously. After observation for one month, we analyzed correlation between individual health condition and their ophthalmic max temperature.

Results: In results of tests in several farms, we confirmed that our system can clearly distinguish individuals in each group and measure their ophthalmic max temperatures automatically and stably by using the infrared thermography during drinking milk. In addition, our data indicated that the measured max temperatures were strongly reflected by animal health condition. Moreover, this system has also a function that correct ophthalmic max temperature according to daily fluctuation of ambient temperature by monitoring ambient temperature of animals continuously. Therefore, we can accurately understand individual normal temperature without consideration of ambient temperature of animals by using our system.

Conclusions: Our system would enable to early detection and effective treatment of affected animals by monitoring their body temperature using infrared thermography. Therefore, it would contribute to reducing animal suffering and improving the economic losses in farm.

Herd Health Management: Dairy

P02-002-100

A participatory on-farm approach leads to the identification of farm-specific management actions and high implementation rates in organic dairy farms

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Objectives: In terms of production diseases a considerable proportion of organic dairy farms in Europe do not meet the organic principle of good animal health. Main reasons for the great variation in animal health under organic conditions are different management practices, restrictions in the availability of resources, and a lack of control mechanisms. Numerous studies have concluded that farm-individual approaches involving the necessary expertise and taking into account system dynamics and farm-specific opportunities and constraints are

needed to identify effective measures that are likely to be implemented, both in organic and conventional farms.

Materials and Methods: Organic dairy farms participating in the IMPRO project (impro-dairy.eu) were visited once in 2013 for an assessment of general characteristics, resources, and management procedures. A second visit was carried out in 2014 to conduct a round-table discussion between the farmer, the farm veterinarian and an agricultural advisor. The second visit, which took between 2 and 4 hours, was moderated by a researcher and followed the same procedure on all farms: farm walk, evaluation of the animal health status, system analysis by an impact matrix, cost-assessment of production diseases, identification of areas in need of improvement, discussion of potential management measures, agreement on suitable actions, feedback session. All farms were surveyed 1 year after the second visit by a written questionnaire to assess the level of compliance with the action plans and evaluate the limitations leading to non-compliance.

Results: In total 192 farms were visited. Due to involvement in other project activities 60, 29, 28 and 32 farms in Germany (DE), Spain (ES), France (FR) and Sweden (SE), respectively were part of the final survey and analysed for this report. In 11 cases (8%) no list of actions was agreed upon while suitable actions were identified in the remaining 138 farms, the number of actions ranging from 1 to 22 with a median (m_{ALL}) of 8. Least actions per farm were identified in FR (m_{FR} 2.5) and most in SE (m_{SE} 12). Actions ranged from very general ('improve feeding') to very specific ('use dry-cow therapy in case of positive California Mastitis Test'). The survey response rate was 63.1%. Of the 94 farms that responded, 88 (93.6%) had implemented at least one measure. The number of implemented measures ranged from 0 to 17 and was lowest in FR (m_{FR} 1) and highest in SE (m_{SE} 7). The rate of implementation was highest in FR (m_{FR} 81.7%) and 66.7%, 65% and 18.0% in DE, SE and ES, respectively. Most actions that were not implemented within one year were related to construction. Other limitations were (in order of frequency of response) time constraints, doubts about the effect, excessive costs, absence of the initial problem, implementation of alternative action(s), lack of expertise, and doubts about the appropriateness of the action.

Conclusions: It can be concluded that a structured participatory approach to health planning involving relevant stakeholders and an analysis of the whole farm system, its opportunities and constraints, can result in action plans tailored to the farm-specific situation and likely to be implemented. The limitations emphasise that 1 year is too short to assess the implementation of construction measures. Moreover, they indicate that farmers in Europe are under great economic pressure and that a lack of conviction often keeps them from taking actions to improve animal health. Future research and advisory practice should take these constraints into account.

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Herd Health Management: Dairy

P02-002-101

A study of a bolus system for continuous monitoring of ruminal pH in dairy cows under physiological conditions.

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Objectives: Systems to monitor ruminal pH levels of dairy cows aim to contribute to the general dairy herd health management. Besides the frequently used single point measurement techniques, intraruminal continuous pH measurement systems are focusing in particular on the diagnosis of subacute ruminal acidosis (SARA). However, there is increasing evidence suggesting a considerable variability in measured pH depending on the system used. We tested if the accuracy of continuous pH monitoring systems is sufficient to provide reliable data of the ruminal conditions. The benefits and limits of ruminal pH measurements in terms of validity were elucidated.

Materials and Methods: For the present study a continuous measurement system was used in order to record ruminal pH (smaXtec GmbH, Graz, Austria). According to the manufacturer, the accuracy of the applied pH bolus is ± 0.2 and the resolution is ± 0.01 . To examine the accuracy and validity of the system, two different experiments with either six or eight lactating Holstein Friesian cows equipped with a pH bolus were performed.

In the first experiment, 6 cows with an indwelling pH bolus were fed on a performance oriented grass-maize based Total Mixed Ration (TMR). Ruminal pH levels were continuously monitored. 49 days later each cow received a second pH bolus. On day 50 data from both devices were compared over a period of 24 hours and analysed for differences in daily pH-fluctuation.

In a second experiment, 8 fistulated cows with an indwelling pH bolus were fed on a TMR and additional concentrate supplementation. Again, pH values were continuously monitored. At a given time the cows were exposed to an eight hour lasting feed deprivation inducing an increase of the ruminal pH level. Additionally, the pH bolus from each cow was removed for approximately two hours through the ruminal fistula in order to test the accuracy of measurement by incubation in buffer solutions of pH 5 and 7 (39°C). Simultaneously, pH values were also analysed both by direct measurements of the ruminal fluid near the fistula using a pH meter and by obtaining ruminal fluid through a common suction pump, the pH of which was determined immediately afterwards

Results: Despite the considerable variability of the absolute pH value of the individuals, typical daily fluctuations in pH related to feeding, milking and treatments were observed.

In the first experiment, mean values of the pH during week 1 to 4 differed only slightly and showed expected daily changes according to circadian rhythm and feeding strategy of the farm. From week 5, the measured pH values began to decrease slightly, resulting in a pH drop of approximately 0.35 units (between first and 7th week). Comparison of the first and the second pH bolus on day 50 revealed differences ranging from 0.26 to 0.47 pH units with a mean of 0.39 ± 0.08 . Only one pair of devices yielded values that were comparable (-0.09 ± 0.09). The pH values of the first bolus were significantly lower than those measured by the second bolus.

In the second experiment, the 8 hours feed deprivation resulted in a slight increase of pH. Restarting the feeding decreased pH values by -0.84 ± 0.18 units. These changes occurred within 103 ± 22 min and recovery was reached within 24 ± 11 hours.

Removing the bolus through the cow's ruminal fistula and incubating it in artificial buffer solutions with a known pH revealed considerable shifts in accuracy: At pH 7 an average pH of only 6.60 ± 0.39 was measured, at pH 5 the average pH ranged from 4.58 to 5.61. pH values obtained by direct measurement through a fistula (5.52 ± 0.11) and those obtained in ruminal samples after aspiration (5.67 ± 0.31) differed significantly from

the value measured by the pH bolus in the corresponding time (6.39 ± 0.28).

Conclusions: The continuous pH monitoring system used in the study provides reasonably reliable data on the typical daily ruminal pH fluctuation of dairy cows. However, the accuracy in terms of absolute pH values appears less than optimal, so that the impact of continuous pH measurements on herd health may be debatable. Since the diagnosis of SARA strongly depends on absolute pH values during a certain time period, the data provided by continuous pH monitoring systems should be interpreted cautiously.

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Herd Health Management: Dairy

P02-002-102

Optimization of dry cow management can influence natural antibody (Nab) levels and the incidence of postpartum disorders in dairy cows

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Objectives: Resilience plays an important role in the ability of cows to remain healthy and productive and can be measured by evaluation of immune parameters in cows. In previous research we found that natural antibody levels (NABs) combined with parity, SCC and postpartum disorders in the prior lactation could indicate whether a cow had a low or high risk for developing a disorder in the subsequent lactation. The aim of this study was to evaluate if postpartum disorders could be prevented by management optimisation of dry cows and whether this optimisation resulted in a change in NAb levels and thus in the risk profile of the cows.

Materials and Methods: In 29 Dutch dairy herds milk samples of cows were tested for IgM and IgG NAb levels at each test-day milking from December 2013 to March 2015. From April 2014 on, for each study herd a tailored plan was made to optimize dry cow management by a veterinarian specialized in feed and dry cow management in collaboration with the herd owners. During the optimisation process which lasted until March 2015, the farmers were guided intensively by the veterinarian with at least bi-monthly contact moments (either in person, by phone, app or email).

Besides optimisation, test-day milk recording data, fertility records, identification and registration data and comprehensive data from livestock disease registration systems were available. Digital disease registration contained cow-level records with the disease and the date it was observed by the farmer.

We evaluated whether the amount of postpartum disorders had changed during the period in which optimisation was conducted. A multilevel logistic regression model was used with postpartum disorder as dependent variable and period (before, at the start or during optimisation), parity, quality of optimisation and a random herd effect as independent variables. In addition, a linear regression model was used to evaluate the influence and quality of optimisation on NAb levels during the study period. The quality of optimisation was also used to divide the study herds in three classes, herds that applied all, part of, or none of the optimisation measures as indicated by the veterinarian.

Results: Besides a large variety of herd-specific optimisation measures, three measures could be applied in all herds:

1. Improve intake of dry matter
2. Optimisation of the ration of dry cows

Other optimisation measures were often related to reduction of infection pressure and stress reduction.

During the study period, the percentage of postpartum disorders decreased from on average 21% prior to optimisation to 16% during optimisation (figure 1a). During optimisation cows had a 1.6 (95% CI: 1.1-2.4) times lower probability (odds) to develop a postpartum disorder as compared to the period prior to optimisation.

Figure 1. Percentage of cows with a postpartum disorder (a) and average natural antibody levels (b) in 29 dairy herds, prior to optimisation (before dashed lines (May 2014)).

Higher NAb levels were known to be associated with higher incidence of postpartum disorders. It appeared that the NAb levels of the cows in the study herds decreased significantly in the period in which dry cow management was optimized. The decrease in NAb level was more evident in herds that applied part or all of the optimisation measures compared to herds that did not optimize their management (Figure 1b). Lower NAb levels contribute to a smaller proportion of cows defined as high risk, thus it seemed that the risk profile of cattle for postpartum disease could be changed by optimisation of dry cow management.

Conclusions: Our study showed that it is possible to prevent cows for developing postpartum disorders, from actually getting diseased by optimisation of dry cow management. In addition, by optimising dry cow management the Nab levels in dairy cows decreased resulting in a decreased risk for developing postpartum disorders in the subsequent lactation.

Herd Health Management: Dairy

P02-002-103

Diagnostic Validity Of Real Time Measurement Of Reticular Temperature For The Prediction Of Parturition And Estrus In Dairy Cows

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Objectives: It is proven that the reticular temperature (RT) in ruminants is representative for the body temperature. In present study the suitability of the (RT) as an indicator for expected parturition and estrus of dairy cows was investigated.

Materials and Methods: 25 parturitions and 43 estruses were recorded. Estrus was confirmed by frequent measurement of milk-progesterone and, retrospectively, by a successful artificial insemination. The RT was measured continuously every ten minutes with indwelling reticular sensors and data were read out by telemetry. The average ambient temperature during the study period was 4.43° C (± 7.86) and the mean RT 39.23° C (± 0.33). In this study the averages of RT-day, 5 days before up to 2 days after estrus, the RT-4 hour averages from 48 hours up to 20 hours after the temperature maximum at estrus and the RT-day averages 10 days before up to 10 days after calving, were analyzed. Time of day, feeding, breed and lactation number were also considered.

Results: RT was sign. influenced by time of day and ambient temperature. RT was also sign. affected by the occurrence of estrus. The mean RT on the day of estrus was 0.15° C higher than the day before. The maximum RT-4-hour average on the day of estrus (39.71° C) was also increased sign. The results for heat detection showed an area under curve (AUC) of 0.81. A sign. effect of parturition on the RT was also found. 48 hours prior to calving RT decreased sign. by 0.43° C. No sign. difference was found between one day before parturition and the day of parturition. Up to a temperature threshold of $\geq 0.40^{\circ}$ C, 100% of the parturitions were detected by RT within 24 up to 48 hours, with a specificity of up to 93%. The prediction of a parturition within 24 and 48 hours showed an AUC of 0.99.

Conclusions: We conclude that continuous RT measurement as used herein is highly suitable for detecting upcoming parturitions and, to a lesser extent, to identify cows in heat. Further results are promising for the early detection of health problems linked with an increase or decrease of body temperature.

Herd Health Management: Dairy

P02-002-104

Effect of duration and temperature during the delivery of bovine blood samples to the analytical laboratory on metabolic parameters serum assays

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Objectives: Blood samples collected in bovine medicine are often exposed to a range of environmental conditions prior to analysis in a laboratory. The stability of the molecules in the bovine blood is poorly understood. The aim of this study was to determine analyte stability of blood specimens in serum tubes exposed to a range times and two storage temperatures prior to centrifugation.

Materials and Methods: Blood samples were taken in 10 lactating Montbeliarde cows in one farm, 1.5 hours after the morning diet distribution. 17 blood tubes were taken from the jugular vein of each animal. The samples were immediately placed in a cooler box, protected from light, and sent to the laboratory. For each cow, one tube was centrifuged and analyzed in the 3 hours after collection (D0) as controls. 8 samples, uncentrifuged, were placed in a refrigerated chamber at 4°C and 8 samples, left at room temperature (25°C). All tubes were kept sheltered from the light. The day D1, D2, D3, D4, D7, D8, D9, D10, one tube stored at room temperature and one kept cool tube were centrifuged and analyzed (in duplicate). The analysis methods used were HPLC for vitamin A, E, and β carotene or enzymatic colorimetric method with an automated biochemistry analyser (JEOL Biomajesty 6010 with Biomérieux, Diasys, Diacron and Randox kits).

Statistical analysis was performed with the Excel © software. Outliers were removed by the Grubbs test. For each pair number storage day / storage temperature and each parameter, the averages of the 10 cows was compared with the average to D0 by a Student's t test for paired data if the distribution was normal (checked by Shapiro-Wilk test) and the variances equal (verified by Cochran's test) or by test of Wilcoxon signed rank in other cases. The results of stored samples were considered to be different from the value on D0 (reference) if the difference between average of stored samples and D0 average was greater than the measurement uncertainty or if $p > 0.05$.

Results: Assay results are identical to the D0 value during the numbers of days (d) following : (for samples stored at 4°C and 25°C respectively) : Bile acids : 3 d, 3 d ; nonesterified fatty acids (NEFA) 8 d, 1 d ; pancreatic amylase 8 d, 8 d ; aspartate amino transferase (ASAT) 4 d, 2 d ; β carotene 8 d, 8 d ; conjugated bilirubin 2 d, 1 d ; total bilirubin 2 d, 1 d ; β hydroxybutyrate (BOH) 10 d, 4 d ; calcium 10 d, 7 d ; chloride 8 d, 8d ; cholesterol 10 d, 10 d ; creatine kinase (CK) 4 d, 2 d ; total carbon dioxide 8 d, 8 d ; creatinine 9 d, 9 d ; reactive oxygen species production (d- ROMs, a subclass of reactive oxygen metabolites (ROM) used to determine the antioxidant capacity of plasma) 4 d, 1 d ; iron 9 d, 4 d ; gamma glutamyl transferase (GGT) 4 d, 4 d ; glutamate dehydrogenase (GLDH) 10 d, 7 d ; lipase 2 d, 2 d ; magnesium 10 d, 10 d ; serum total antioxidant capability (OXY-adsorben test, measures the ability of a plasma sample to object to a massive oxidative insult induced in vitro by a solution of hypochlorous acid) 10 d, 10 d ; Alkaline phosphatase (PAL) 10 d, 10 d ; Phosphate Inorganic 10 d, 3 d ; Potassium 1 d, 1 d ; Total Protein 10 d, 10 d ; Sodium 4 d, 4 d ; Total Iron-Binding Capacity (TIBC) 10 d, 10 d ; Triglycerides 4 d, 3 d ; Urea 10 d, 3 d ; vitamin A 10 d, 10 d and vitamin E 10 d, 10 d.

The storage temperature has no effect on the duration of biochemical parameters stability for half of the measured parameters, for the others parameters, the stability is better when the sample is kept cool.

Conclusions: The stability of some biochemical indicator is very long, it reached 10 days (Total Protein, BHB, urea, cholesterol, Ca, Mg, Phosphate Inorganic, PAL, GLDH, ...) especially for vitamin A and E deemed fragile. Very few parameters require analysis rapidly after collection (bile acids, bilirubin, lipase). These results show a very good stability of biochemical parameters in cattle. The use of biochemical assays should not be limited even if the delivery times in the laboratory is several days. However, the laboratory should check the temperature on receipt of samples to ensure the reliability of the value (NEFA, CK, d-ROMs, potassium, urea).

Herd Health Management: Dairy

P02-002-105

Diversity of behavioural patterns displayed by an autumn calved, recently weaned cohort of dairy calves in SE QLD dairy herd

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Objectives: Timely detection of morbid animal has a detrimental effect on animal productivity and animal welfare¹. Movement trends have been extensively studied in wildlife to monitor annual migration and prey-predator interactions.^{2,4} Different species of animals develop distinct movement trajectories that can be monitored overtime and used as indicator of health.⁵ The objective of the study was to describe diversity of the movement patterns displayed by a recently weaned cohort of dairy calves soon after weaning and to assess the feasibility of collecting such data as a proof of concept to monitor dairy calves' movement patterns.

Materials and Methods: This pilot cohort study was conducted on dairy calves at the University of Queensland, Gatton campus, South East Queensland in September 2015. There were 14 Holstein calves comprised of six males and eight females. The age range for these calves was 8-9 weeks old. The calves were fitted with GPS data loggers (Igot-U GT600, Mobile Action Technology Inc, Taipei, Taiwan) to capture coordinates, distance, time and speed. Each calf was identified by an ear

tag. The combination of ear tag and GPS unit serial number was used as a form of identification during the study. The data loggers were pre-set to record data at twenty seconds interval over a twenty four hour period for the duration of three days. This interval reduced chances of omitting little movements.⁶ The following variables were calculated to describe the movement patterns of the calves; distance, rate of movement (ROM), angles of turns and linearity ratio.⁷ The mean and standard deviation were calculated for all the variables, excluding linearity ratios. A within group sum of the squares plot was used to identify clusters in the trajectory data set. A k-means cluster analysis was used to divide relocations into one of the identified clusters. Univariate analyses of variance (ANOVA) were used to test for the significance of each matrix variables in characterising calves movement pattern. Analyses were done using the fpc⁸ and adehabitat packages⁹ in the R package.

Results: Overall, there were 17532 relocations made by the 14 calves with 618 being the smallest number of relocations. The highest number of recorded relocations was 3725. There was a significant difference (P<0.001) in the number of relocations made by the individual calves. There were eight calves that recorded over 1000 relocations each against only three registering relocations less than 1000 relocations. The number of relocations at about 250 were recorded at 6pm with the next other highest number of relocations captured at 8 and 9pm. The highest numbers of relocations occurred between afternoon and night (P<0.01). Three distinct movement patterns were identified using cluster analysis: a) the foraging pattern cluster characterised by short distance movements over a wide geographic area with big turning angles; b) travelling pattern cluster which is associated with large distance movements with small turning angles and high speed; and c) the resting or bedding pattern cluster.

Conclusions: There was a significant difference in the number of relocations and movement patterns made by the individual calves. Significant movement activities from the study animals occurred mostly in the afternoon and at night. Three distinct movement patterns have been identified with significant differences in distance travelled, rate of movement and linearity ratios. This study shows that despite the complexity of animal movement behaviours collecting movement data is feasible and could potentially be developed further to enhance remote monitoring of health and wellbeing of dairy calves.

Herd Health Management: Dairy

P02-002-106

Evaluation of quality and management of colostrum on dairy farms in Spain

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Objectives: The quality and management of colostrum is the most important risk factor for the health of the dairy calf. However, to our knowledge no previous study has evaluated the quality and management of colostrum on Spanish dairy farms. The objective of this study was to evaluate colostrum quality and frequency of failure of passive transfer of immunity to newborn calves.

Materials and Methods: Twenty five dairy farms located in different regions of Spain were selected. In each farm a three step program was implemented: 1) epidemiological questionnaire including main critical points for colostrum management on farm; 2) sampling of colostrum (a minimum of five colostrum samples per farm) and sera from newborn calves (a minimum of five calves of 24h to 6 days old per farm); and 3)

individual and overall analysis of the results and recommendations to improve colostrum management.

The samples of fresh, frozen or refrigerated colostrum were collected just before the first administration to the newborn calf, according to normal on farm practices. Then, the colostrum was analyzed to determine its composition (fat, protein, somatic cell count (SCC)); microbiological quality (total plate count (TPC) and coliform count (CC)); and immunological quality (IgG concentration, mg/mL). In addition, serum samples were taken from calves. Whenever possible, the sampled calves were the ones previously fed the sampled colostrum. The serum was analyzed to evaluate FPT through direct measure of IgG (g/mL) and also indirectly by total proteins (g/dL) and Brix refractometry (%).

Results: This information will be very useful to identify the proportion of colostrum that meets the minimum standard IgG concentration (50 mg IgG/mL in colostrum); and also to calculate the quantity of colostrum we should recommend to feed to the calves in order to reach the necessary IgG absorption (10 mg IgG/mL in calves sera to avoid FPT. On the other hand, it will allow an evaluation of the colostrum management on farm mainly based on: the level of contamination (bacteriological count), the milligrams of IgG administered in first colostrum feeding (based on its quality, quantity and time of administration) and the prevalence of FPT in newborn dairy calves (1-7 days old).

As the study is still in process, the final results and conclusions will be completed and sent to the WBC organization by February 2016 and presented at the congress.

Conclusions: This information will be very useful to identify the proportion of colostrum that meets the minimum standard IgG concentration, calculate the quantity of colostrum recommended in calves sera to avoid FPT. On the other hand, it will allow on farm colostrum management evaluation mainly based on: the level of contamination (bacteriological count), amount of IgG administered in first feeding and the prevalence of FPT in newborn dairy calves (1-7 days old).

As the study is still in process, the final results and conclusions will be completed and sent to the WBC organization by February 2016 and presented at the congress.

Herd Health Management: Dairy

P02-002-107

Metabolic status of F1 Holstein x Gir dairy cows during transition period in two different seasons

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Objectives: Brazil has a seasonal milk production due to differences in the climate and food supply. In Minas Gerais the summer period is hotter and rainy with higher grazing availability but heat stress can be very severe. In winter the temperature are mild and there is almost no rain. In this tropical condition the crossbreed F1 Holstein x Gir is proving to be a good alternative for milk production. Thereafter, our works focus on clarifying the metabolic pattern of F1 Holstein x Gir dairy cows during the most critical time in the lactation cycle, the transition period and compare their performance in two seasons.

Materials and Methods: The research took place at a dairy farm located in Minas Gerais State with a herd of 120 lactating cows producing 2,000

liters of milk/ day. We took blood samples from 15 cows during summer (January to April) and 13 cows during winter (May to August). All cows were crossbred ½Holstein ½ Gyr, pluriparous, with lactation order from second to the fifth with average daily milk production of 24L in both seasons. Cows remained in mixed paddocks and received total mixed ratio of concentrate and corn silage twice a day. The Ethics Committee on Animal Experimentation of Universidade Federal de Minas Gerais approved the project.

We took blood samples by puncture of the coccygeal vein weekly during the prepartum beginning in the fourth week before calving expected date, one sampling 24 hours after calving, and in the second, fifth, 10th, 15th, 21th and 30th days postpartum. Thereafter, we conducted analyses of triglycerides, cholesterol, glucose, gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) using the technique of spectrophotometry in automatic device and commercial kits.

We used cutoff points of 1.2 mmol/L of BHB for subclinical cetose diagnose and 0.4 mmol/L of NEFA in prepartum and 0.7 mmol/L in postpartum for animals with higher risk for diseases.

The design was a split plot arrangement in which the seasons consisted of plots and times of blood collection the subplots. We used Scott-Knott test for time of assessment and by SNK test for comparison between seasons.

Results: The season had a great influence in the metabolic status seeing that only AST, NEFA and BHB had no variation. Glucose was higher in winter during the whole period. LDH was higher in summer during postpartum. Cholesterol was lower in winter than in summer during the first week prepartum and calving day.

Sometimes, more important than the difference in concentration is the difference in the patten of the metabolites during the transition period between the seasons. On this aspect, almost all evaluated metabolites had a different patten with the exception of glucose, NEFA and BHB.

The mean concentration of NEFA and BHB never reached the cutoff points, but a high number of cows had experienced subclinical cetose or had concentrations of NEFA above the limit. In total, 40.0% of animals had subclinical cetose and 46.67% had high levels of NEFA in winter, while in summer 53.85% of animals had subclinical cetose and 30.77% had high levels of NEFA. It is important to note that the frequency of animals above cutoff points for NEFA and BHB does not follow the same pattern of the mean concentration. This means that the data have to be analyzed by the two ways.

Regarding the sampling time, all liver enzymes and cholesterol increased their activity and concentration after calving while glucose and triglycerides concentrations decreased due to the use for milk synthesis. NEFA concentrations increased one week before calving, reached 0.45 mmol/L at 5th day and decreased 10 days after calving. BHB concentrations increased on the calving day reaching their peak of 0.90 mmol/L on the 5th day.

Conclusions: The metabolic profile of F1 Holstein x Gir cows in semi-confined system differ greatly between summer and winter, reflecting the great difference especially in environmental conditions.

The metabolic status of crossbred ½Holstein ½ Gyr dairy cows in the transition period appeared very similar to the patten presented by cows with a high Holstein composition in similar conditions. This may be due to the genetic selection and consequently increased milk production.

An alarming number of cows had experienced subclinical cetose and high NEFA concentrations, showing that this animals had a greater chance for developing other diseases.

Herd Health Management: Dairy

P02-002-108

Genetic characterization of antimicrobial resistance in *Staphylococcus aureus* cultured from bovine mastitis cases in Northwest China

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Objectives: *Staphylococcus aureus* is the most common etiological pathogen of bovine mastitis. The resistant strains make the infections difficult to treat. The objective of the present study was to characterize the genetic nature of the antimicrobial resistance-encoding genes in *S. aureus* cultured from bovine mastitis in Northwest China in 2014.

Materials and Methods: A total of 44 *S. aureus* were isolated in this study. These included 32 strains isolated from clinical cases and 12 strains isolated from subclinical cases. The regions and number of isolates included Gansu (23), Qinghai (11) and Ningxia (10). Mastitis infection was confirmed by California Mastitis Test (CMT). Milk samples were collected aseptically for bacteriological assay. Before sampling, the first streams of milk were discarded, and teat ends were disinfected with cotton swabs soaked in 70% [v/v] alcohol and allowed to dry. Then a 5 mL secretion was collected into a sterile 10 mL tube. Any samples that were not processed immediately were kept at 4 °C for microbiological examination within 18 h of collection.

Results: Susceptibility to a panel of antimicrobial compounds was determined by disc diffusion and the corresponding resistance-encoding genes were detected by polymerase chain reaction (PCR). Phenotypic resistance among these isolates was found to penicillin (84.09%), erythromycin (20.45%), tetracycline (15.91%), gentamicin (9.09%), tobramycin (6.82%), kanamycin (6.82%), and methicillin (2.27%). In total, 9.09% of the *S. aureus* isolates were classified as multidrug resistant. In addition, the genotypes underpinning resistance to rifampicin (100%, rpoB), penicillin (95.45%, blaZ), tetracycline (22.73%, tetK, tetM, alone or in combination), erythromycin (22.73%, ermB or ermC), gentamicin/tobramycin/kanamycin (2.27%, aacA-aphD), methicillin (2.27%, mecA) and vancomycin (2.27%, vanA) were also identified. Resistance to tetracycline was attributed to the genes tetK and tetM ($r = 0.558$, $P < 0.001$).

Conclusions: Thus study noted high-level geno- and phenotypic antimicrobial resistance in *S. aureus* isolates from bovine mastitis cases in Northwest China and confirmed that genotype resistance cannot be used as a diagnostic indicator for phenotypic antimicrobial susceptibility patterns.

Herd Health Management: Dairy

P02-002-109

The effect of gradual weaning and breed on the blood mRNA transcriptome of artificially reared Holstein-Friesian and Jersey calves

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Objectives: Holstein-Friesian and Jersey are the predominant dairy breeds in Ireland. Variance in haematological profiles, expression of genes implicated in immune responses and in vitro neutrophil activity has been observed between these calf breeds. Weaning was reported to induce a pro-inflammatory stress response in abruptly weaned beef calves. However, limited literature is available on the dairy calf immune response to weaning. Therefore, the objective of this study was to examine the effect of (i) gradual weaning and (ii) breed, on the whole blood mRNA transcriptome of artificially-reared Holstein-Friesian and Jersey calves using RNA-Seq.

Materials and Methods: Holstein-Friesian (n=8) and Jersey (n=8) bull calves were group housed and individually fed milk-replacer and concentrate, using an automatic feeder, for 56 days. During the pre-weaning period, calves were offered 0.8 kg milk replacer (6 litres (l) at 133.33 g/l) and ad libitum concentrate. Prior to weaning (day (d) 0), milk-replacer was gradually reduced over the preceding 13 days (d -13 to d -1) and by d -1 all calves were consuming at least 1 kg of concentrate per day for three consecutive days. On d 0, milk-replacer was eliminated from the diet of all calves. Calves were blood sampled into Tempus Blood RNA Tubes on d -14, 1, and 8, relative to d 0. Total RNA was extracted from whole blood. Forty-eight individual libraries were prepared, validated, pooled, and sent to Clinical Genomics (Canada), for RNA sequencing on an Illumina HiSeq. Forty million 76 bp paired end reads were sequenced per library. Raw sequence reads were assessed for quality using FastQC. Reads were aligned to the bovine genome UMD3.1 using the STAR aligner. Read counts were generated using htseq-count. Differential gene expression was determined using EdgeR. Pathway and downstream biological functional effects analyses were performed within Goseq using Kyoto encyclopaedia of genes and genomes (KEGG) and Ingenuity® Pathway Analysis, respectively, on statistically significant (P<0.05) differentially expressed genes (DEGs), with a false discovery rate (FDR) of 10% and a fold change of ≥1.5. A 10% FDR cut-off was implemented on the results of the pathway analysis using Bioconductor's qvalue package.

Results: There were no DEGs in response to gradual weaning (i.e. within breed between blood-sampling days). The data therefore suggests that gradual weaning of artificially-reared dairy calves does not compromise their welfare. At a false discovery rate of 10% and with a ≥1.5-fold change, 550 DEGs were detected between Holstein-Friesian and Jersey calves on d -14, 490 DEGs on d 1, and 411 DEGs on d 8, respectively. Nine, five and five, KEGG pathways were over-represented among the DEGs between the two breeds on d -14, d 1, and d 8, respectively (P<0.01; Q≤0.1). The cytokine-cytokine receptor interaction and the neuroactive ligand-receptor interaction pathways were over-represented between breeds on all days (P<0.01; Q≤0.1). These pathways contained immunological genes down-regulated in Jersey relative to Holstein-Friesian calves which are involved in cell signalling and monocyte and T lymphocyte activation, chemotaxis and phagocytosis. The results of the downstream biological functional effects analysis also suggested decreases in cell signalling activity along with chemotactic and phagocytic functions for Jersey relative to Holstein-Friesian calves, on all days. Therefore Holstein-Friesian and Jersey calves differ in transcription of many immune response genes under artificial rearing conditions without exposure to experimental challenge with pathogens.

Conclusions: In conclusion, the data suggest that gradual weaning of artificially-reared dairy calves does not compromise their welfare, evidenced by the lack of expression changes in genes involved in immune and/or stress responses. Additionally, these results demonstrate

transcriptional down-regulation of genes involved in cell signalling and immune responses in Jersey compared with Holstein-Friesian calves. Knowledge of breed-specific immune responses could enable improved health management practices which could be better tailored towards the specific disease sensitivities of Holstein-Friesian and Jersey calves.

Herd Health Management: Dairy

P02-002-110

Relationships between rumination pattern, time of calving and drenching in dairy cows

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Objectives: The objective of this study was to monitor the rumination pattern during the peripartur period in pluriparous dairy cows. The study also aimed to examine the effect of postpartum drenching on rumination times.

Materials and Methods: The study was carried out on a commercial dairy farm and involved 63 Hungarian Holstein Frisian cows. The rumination time was continuously recorded by using an automatic system (Hr-Tag, SCR Engineers Ltd., Netanya, Israel), which were placed on the cows approximately 2 weeks before calving and they were removed 4 days after calving. The data were calculated and summarized in 2-hour intervals. The postpartum drenching (D) was performed on 32 cows randomly, and the control group (C) consisted of 31 cows. The time of calving was recorded in 44 cows. Cows in the D group were drenched with a nutritional supplement consisting of 14% calcium, 42% propionate, 6% magnesium, 6% potassium, 3% sodium, 9% chloride and 20% yeast solved in 25 liters of lukewarm water,. Drenching was performed three times approximately 24 hours apart. The exact time of the drenching was recorded. The first drench had to be done in the first 24 hours after calving. Cows in the group C were handled exactly the same way except for the drenching.

Results: There is a tendency toward a decreased rumination time from 8 to 10 hours to 6 to 8 hours before calving (17.2%, P=0.055). Rumination time decreases significantly from 6 to 8 hours before calving to 4 to 6 hours before calving (45.5%, P<0.001), and another significant decrease could be detected between 4 to 6 hours and 2 to 4 hours before calving (58.5%, P<0.001). A non-significant decrease could be found in the 2-hour interval in which the calving proceeded compared to the previous interval (2 to 4 hours before calving). The rumination time resumed the values prior to the first decrease in 24 to 28 hours after calving. After summing up the daily rumination times, a strongly significant decrease was detected on the day of the calving compared to the previous day (P<0.0001). The rumination times of the cows in the D group significantly decreased at the time of drenching (37%, P<0.05) compared to the controls. The difference could also be detected in the next 4 to 6 hours (P<0.05).

Conclusions: We can conclude that the automatic measurement of rumination time is useful to predict the time of calving both on daily and hourly level. Drenching, however, doesn't seem to have a positive effect on rumination times immediately after the treatment.

Herd Health Management: Dairy

P02-002-111

Modeling Lactation Curve For Milk Fat To Protein Ratio In Holstein Cows

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Objectives: The aim of this study was to examine seven empirical mathematical models (Brody, Wood, Dhanoa, Sikka, Nelder, Rook and Dijkstra) currently used to fit 305 day standard lactations to evaluate their efficiency in describing the lactation curve for milk fat to protein ratio (FPR) in the first three lactations of Iranian Holsteins.

Materials and Methods: Data were 8,103,044 test-day records for FPR from the first three lactations of Iranian Holstein cows which were collected on 2392 dairy herds in the period from 1991 to 2014 by the Animal Breeding Center of Iran. Each model was fitted to monthly FPR records of dairy cows using the NLIN and MODEL procedures in SAS and the parameters were estimated. The models were tested for goodness of fit using adjusted coefficient of determination, root means square error, Durbin-Watson statistic (DW), Akaike's information criterion (AIC) and Bayesian information criterion (BIC).

Results: The highest peak of FPR was observed in early lactation, which was followed by a decline as the lactation progressed and an increase toward the end of the lactation period. For the first three parities, Dijkstra model provided the lowest AIC and BIC values, but Nelder model had the greatest values of AIC and BIC for the first three parities. Therefore, Dijkstra equation provided the best fit of FPR for the first three parities of Holstein cows, but Nelder model provided the worst fit. According to DW values, it seems that there was only a slight or no problem of residual autocorrelation for FPR in this study.

Conclusions: The change in FPR over the lactation might be an appropriate selection criterion in order to improve the energy status. Evaluation of the different models used in the current study indicated that non-linear functions were adequate for fitting monthly FPR records of Holstein cows.

Herd Health Management: Dairy

P02-002-112

Effect of Ultrasonographic Lung Lesions on Milk Production in Adult Holstein Dairy Cattle

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Objectives: Immune system suppression related to parturition and the onset of lactation increase the susceptibility of dairy cows to infectious disease such as pneumonia. Thoracic ultrasound is a practical means of identifying lung lesions which have been associated with poor performance in dairy calves. Currently, there is a paucity of research evaluating the impact of pneumonia on future performance in lactating dairy cattle. The objectives of this study were to investigate the prevalence of ultrasonographic lung lesions and their impact on milk production in recently fresh Holstein cows.

Materials and Methods: The study was conducted April to October 2015 on a commercial dairy with a high historical rate of pneumonia (12% in 2014) in Wisconsin, USA. Fresh Holstein dairy cows (n=554) were enrolled in the study during twice-weekly farm visits. At each visit, a thoracic ultrasound scan was performed once per cow between 4-7 days in milk (DIM). A portable variable frequency linear ultrasound scanner was used to scan the 10th to 4th intercostal spaces on both sides of the thorax. A score (US score) was assigned based on the ultrasonographic finding, as follows: 0) only normal lung tissue visualized, 1) lobular pneumonia in one or more lobes (limited area of focal consolidation involving less than an entire lobe), 3) lobar pneumonia (one or more lobes entirely consolidated). Individual daily milk weights for each cow were recorded and 60 day milk yield was calculated. Blood β -hydroxybutyrate (BHBA) concentration of each cow was recorded between 7-10 DIM. A fixed effects linear regression model was fit using backwards elimination and commercially available software. The primary predictor of interest was US score and the primary outcome was 60 day milk yield. The final model also included parity (parity 1 and parity 2+), treatment for illness in the first 65 DIM, BHBA, and parity*US score interaction. Clinical cases of pneumonia were treated according to farm protocols by farm staff that were blinded to ultrasound status. This study was approved by the University of Wisconsin Institutional Animal Care and Use Committee.

Results: Nine of 554 cows (1.6%) were treated for clinical pneumonia by farm staff. Thirteen of 554 cows (2.4%) were diagnosed with ultrasonographic lobar pneumonia. The prevalence of lobular lesions was 64% (n=353). Approximately one third (34%; n=188) had no ultrasonographic lesions. Two of the 13 cows with lobar pneumonia were treated for clinical pneumonia by farm staff. Conversely, 2/3 cows that were treated between 4-7 DIM for clinical pneumonia did not have lung lesions. In general, 60 day milk yield was greater for parity 2+, than cows in their first lactation (P < 0.001). The effect of US score on 60 day milk yield differed by cow parity (P = 0.02). First lactation cows with lobar lesions had a lower 60 day milk yield (3217 lb, SE 404 lb; n=3) than those with normal lungs (4125 lb, SE 65; n=92) or lobular lesions (4270 lb, SE 76 lb; n=132). Sixty day milk yield was not affected by lung lesions in parity 2+ cows. Other factors impacting 60 day milk yield included treatment for illness and hyperketonemia. Cows treated for an illness produced 230 lb, SE 70 less milk during the first 60 DIM than untreated cows (P = 0.001). Sixty day milk yield increased as BHBA increased, up to 2.9 mmol/L, after which it decreased (P < 0.01).

Conclusions: To the authors' knowledge, this is the first study to demonstrate the negative impact of ultrasonographic lung lesions on milk production and highlights the difficulty in diagnosing pneumonia, as cows with lung lesions were not consistently identified by farm staff. Such discrepancies result in failure to initiate appropriate therapy or the unnecessary treatment of cows lacking lung lesions. This is particularly important in light of recent concerns regarding proper drug use, antimicrobial resistance, and food safety. Future studies should evaluate the impact of treating cows with ultrasonographic lung lesions on milk yield.

Herd Health Management: Dairy

P02-002-113

A dairy farm simulation model as a tool to explore the technical and economical consequences of management decisions

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Objectives: Dairy farms are facing difficulties due to high feeding costs, and low milk prices and profits. The need to improve the competitiveness of the dairy enterprise requires decisions that are often difficult to evaluate because they are highly dependent on many economic and technical factors and their interactions. These complex interactions can only be predicted by mathematical modeling. The objective of the present project is to present a stochastic mathematical model to simulate the functioning of a dairy farm, and to evaluate the impact of changes in one or several technical or economic factors on its profitability.

Materials and Methods: The model is fed with data from a dairy farm or using of a randomly generated farm under the specifications defined by the user. The model accounts for heifers, lactating and dry cows (biological model for each group). For each animal, sub models are developed for feeding, diseases, environmental factors, facilities, management, reproductive management, and economics. All these factors follow established equations that account for the complex interactions among them. Default values for variables are provided, but the user can changed them. The conditions established are simulated on an animal-by-animal and day-by-day basis. The outcome provides a list of technical and economic indicators essential for the decision-making process, including demographics, production, reproduction, heifers, diseases and economics. As an example, a 300 adult dairy cow farm (average of 38 L/milking cow/day) with the replacement heifers was used. The farm had an average pregnancy rate of 15%, a milk price of 0.33 euros/L and a labor efficiency 43 cows/person. The question was raised on the suitability of using a time-fixed artificial insemination protocol. The timed-fixed insemination was planned only for the first insemination at 77 DIM, with a 50% fertility for first lactation cows, and 45% fertility for older cows, and a cost of 13 euros per treatment. The model was used to provide the proper data to help in the decision process.

Results: During the 4-year simulation, the application of the fixed-time artificial insemination reduced DIM (from 202 to 181 days) and the proportion of problem cows (not pregnant by 150 DIM; from 14.3 to 8.5 %); and it increased milk production (from 37.4 to 38.6 L/milking cow/day) and the gross margin (from around 54.000 to 70.000 €/year, or from 184 to 236 €/cow/year). The simulation information indicates that for each percentage point improvement in pregnancy rate there is an increase in 4.83 euros in the gross margin per cow. The breakeven point for the use of time fixed artificial insemination was set at a pregnancy rate of 24.2%. The decision was independent of the price of milk over a range of 0.28 to 0.38 €/L or the level of production (tested at 8000 and 15000).

Conclusions: The model provides a powerful and useful tool to explore the medium and long term consequences of changes in technical and economic decisions on the economic sustainability of dairy farms. The program is user friendly, web-based and available in English, Spanish and Catalan (www.dairyfarm.es/simulador).

Herd Health Management: Dairy

P02-002-114

Resilient Cattle; predicting postpartum disease before drying off

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Objectives: Udder health, fertility and leg problems are of importance in the dairy industry and a long productive life of cows. The immune system is a driving force in the maintenance of a good health status. Immune parameters will be evaluated for their capacity to identify resilient individuals. Previous research showed that innate immunity is associated with disease risks in cows, but it is unknown if the occurrence of future diseases can be predicted based on innate immunity parameters. Therefore, the aim of this study was to investigate whether it is possible to predict postpartum disorders using natural antibody levels (NABs) in dairy cattle.

Materials and Methods: In 29 Dutch dairy herds milk samples were obtained from all lactating cows between April 2012 and April 2013. IgM and IgG levels against Keyhole Limpet Hemocyanin (KLH) were determined using an ELISA test (Greiner®). Nabs for KLH are considered to represent the innate immunity level of the cow. Test-day milking recording data, fertility records, identification and registration data and comprehensive data from livestock disease registration systems were available. Digital disease registration contained cow-level records with the disease and the date it was observed by the farmer. Survival analyses techniques were used to evaluate if NABs in the last available milk sample prior to drying off were associated with the time to occurrence of postpartum disorders in the first 60 days of the subsequent lactation. The following postpartum disorders were considered: retained placenta, (endo)metritis and ketosis.

Results: The best final model included IgM NAb level, difference in IgG NAb levels between two measurements, parity, SCC and postpartum disease in prior lactations. The validation data showed that the model classified 32% of the cows as having a high risk for developing postpartum disorders. Of the cows predicted to be at high risk of postpartum disease, 31% developed a postpartum disorder. Of the cows that were classified as having a low risk, 86.1% did not develop a postpartum disorder. This means that almost 1 in 3 cows predicted to get the disease did actually get postpartum disease. This is comparable to the number of cows with high somatic cell count developing mastitis.

Cows that were classified as high risk by the model had a 2,7 times higher odds (95% CI: 2,2-3,3) for developing a postpartum disorder than low risk cows.

Conclusions: The study showed the possibility to predict postpartum disorders with a model that, amongst other variables, contained NABs in milk samples. The model can be used to inform farmers which cattle have a high risk for developing a postpartum disorder. This may assist farmers to take measures to prevent the disorders from occurring.

Herd Health Management: Dairy

P02-002-115

Implementation of vaccination strategies on British dairy farms: the importance of the farmer-veterinary surgeon relationship

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Objectives: Vaccine use in the cattle industry appears to be widespread but there is limited published guidance or set protocols for their use.

Veterinary surgeons have been identified as important sources of advice on disease control and vaccination by farmers, as well as being their preferred vaccine provider. However, there is limited research exploring the farmer-veterinary surgeon relationship in the context of vaccination decision-making.

The objective of this study was to explore the farmer-veterinary surgeon relationship in the context of vaccination decision-making.

Materials and Methods: This study is reported following the Consolidated Criteria for Reporting Qualitative Research (COREQ) guidelines. Twenty-four semi-structured interviews were carried out with dairy farmers, and fourteen with veterinary surgeons. The audio recordings were transcribed verbatim by external transcribers. Anonymised transcripts were imported into qualitative data analysis software (NVivo 10, QSR International) for thematic analysis. To assess the robustness of the coding framework a sample of the transcripts were coded independently by a second researcher.

Results: Thematic analysis suggested that both veterinary surgeons and farmers have a positive attitude towards the use of vaccination and have few barriers to its implementation providing there is evidence of a need to do so. Currently in Britain cattle vaccination schedule decision-making is undertaken on an individual farm basis by farmers and their veterinary surgeon. Farmers perceived their veterinary surgeon to have an important role throughout the decision-making process. The advice of veterinary surgeons was trusted. Veterinary surgeons appeared to group farmers into three 'types' of farmer and these groups influence the vet-farmer relationship and communication. There were differences between farmers and veterinary surgeons extending from differences in risk perception to the perceived influence of outside sources. These differences potentially acted as barriers to effective communication and could therefore lead to either vaccines not being implemented or the use of vaccines that were not necessarily required.

Conclusions: A need for methods to increase farmers' awareness of their herd's disease status and solutions to provide more time and resources to enable vets to discuss disease prevention and control with clients was also highlighted by this study.

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Herd Health Management: Dairy

P02-002-116

Evaluation of heat stress conditions at cow level inside a dairy barn

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Objectives: The precise measurement of climate conditions that cows are exposed to inside a dairy barn is crucial, because already small variations in THI can negatively influence dairy cows.

Besides the housing conditions also the social hierarchy affects the actual experienced heat stress by individual animals because cows actively seek comfortable climate conditions.

The objectives of this study were to examine heat stress conditions

dynamically at cow level, to investigate the relationship to the climate conditions at stationary locations inside a dairy barn and to compare the climate conditions at cow level between primiparous and multiparous cows.

Materials and Methods: The study was conducted on a commercial dairy farm in Sachsen-Anhalt, Germany from May 2014 to July 2014. The herd consisted of 1,200 Holstein dairy cows with an average milk production of 10,147 kg. The barn was positioned in a NE-SW orientation with open ventilation and a mechanical fan-system. Ambient temperature and relative humidity were recorded using EL-USB-2+ data loggers (Lascar electronics, Salisbury, UK) and used to calculate the THI according to the equation reported by the NRC (1971):

$$THI = (1.8 \times AT + 32) - ((0.55 - 0.0055 \times RH) \times (1.8 \times AT - 26)).$$

Days of heat stress were defined as days with a mean THI \geq 72. Stationary climate conditions within the barn were recorded on 2 locations within the milking parlor and on 3 locations within the experimental pen. Climate loggers within the milking parlor were located in the holding area and the rotary parlor. Climate loggers within the experimental pen were secured on an alley position, on a central position, and on a window position located nearby a ventilation opening in the outer wall. Climate conditions at cow level were recorded with climate loggers attached to the collar of the cows within an isolated rubber tube. These climate data were recorded on cohorts of 6 to 10 primiparous and multiparous cows, respectively in 7 replicates for one week each. Multiparous cows were housed inside the experimental pen for a minimum of 3 weeks before each replicate and primiparous cows were transferred to the experimental pen at the first day of each replicate to enforce a subordinate rank in the social hierarchy.

Results: Sixtyone primiparous and 62 multiparous cows were enrolled in the study. A total of 34,657 time values (2 minute intervals) from 52 experimental days was collected. The AT and THI differed significantly between all stationary loggers. The lowest AT and THI was measured at the window logger in the experimental pen and the highest AT and THI was measured at the central logger in the experimental pen. The RH measured at the pen loggers was 4.53% ($P < 0.05$) lower than measured at the milking parlor loggers. The highest RH was measured at the holding area logger and the lowest RH was measured at the window logger in the experimental pen.

The AT measured at the mobile cow loggers was 1.56 °C ($P < 0.05$) higher than measured at the stationary loggers. The THI at the mobile cow loggers was 2.33 THI points ($P < 0.05$) higher than measured at the stationary loggers. Furthermore, the mean daily THI was higher at the mobile cow loggers than at the stationary loggers on all experimental days. Number of days averaging THI \geq 72 were 12.86 percentage points higher measured at mobile cow loggers than at stationary loggers ($n = 427$, $P < 0.05$). There was no significant difference for the AT, RH, and THI between primiparous and multiparous cows.

The THI at the pen loggers was 0.44 THI points ($P < 0.05$) lower when the experimental cow group was located inside the milking parlor. The THI measured at the mobile cow loggers was 1.63 THI points ($P < 0.05$) higher when the experimental cow group was located inside the milking parlor.

Conclusions: Our results indicate, that the actual heat stress experienced of dairy cows differs significantly from the heat stress conditions measured at stationary locations inside the barn and heat and humidity are not eliminated effectively from the cows immediate surrounding. Thus, a wide range of microclimates between different locations as well as between individual cows exists inside a dairy barn. Heat stress is underestimated when climate conditions are obtained from one stationary location inside the barn. For the determination of specific THI thresholds, the climate conditions should be obtained in the immediate surrounding of the cows.

Herd Health Management: Dairy

P02-002-117

Swelling at the injection site after Q fever vaccination - an important information

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Objectives: The zoonotic pathogen *Coxiella burnetii* (Cb) causes Q fever in humans and Coxiellosis in many species of animals of which ruminants are of major concern. Large quantities of Cb are shed during parturition; additionally, individual cows are shedding Cb with their milk. Since 2010 a vaccine (Coxevac[®], CEVA, Düsseldorf) got registered in Germany. It is an important tool to reduce Cb-shedding. An increased awareness among farmers and veterinarians regarding side effects of vaccination exists, because former vaccines induced unacceptable swellings: The size was sometimes comparable to a loaf of bread.

Materials and Methods: In this study 80 dairy cows and their off-springs in a chronically Cb-infected herd were vaccinated. Before vaccination individual milk samples from each quarter of the udder were analyzed for phase-specific antibodies (Phase I and Phase II-ELISA) and by quantitative PCR. At each vaccination a Li-heparin and a clotted blood sample were collected to assess the Interferon-(IFN)- γ -response (IFN- γ -recall assay) and phase-specific antibody titers, respectively. After vaccination (1-3 days p.v.) the injection site was controlled for swelling (diameter in cm) and the temperature was recorded rectally. Pregnancy was controlled by rectal palpation before and after vaccination. Cows in the last month of pregnancy were vaccinated after calving.

Results: In 11 of 72 cows Cb was detected in milk samples. A variable number of quarters of the udder per animal were affected. 47% of animals showed a palpable swelling after 1st vaccination, and in 16% of animals it even exceeded 5 cm. However, they disappeared without treatment within two weeks. Temperature exceeded 39.5°C in 6.1% of vaccinated animals. No abortion was observed.

After 1st vaccination swellings were significantly associated with positive IFN- γ -results (cut-off 15%) before vaccination. Generally, vaccination resulted in a strong increase of Phase II-titers and IFN- γ -reactivity and only a slight increase of Phase I-titers. However, a weak IFN- γ -response and no side effects after 1st vaccination were observed in cows with elevated phase I-titers and detection of Cb in milk before vaccination (i.e. chronic infection of the udder). Remarkably, strong IFN- γ -reactivity in absence of antibodies characterized the immune response of unvaccinated heifers and calves. It is assumed to be due to an early infection of calves. Thus absence of antibodies should not be misinterpreted as non-immune (i.e. susceptible to infection).

Conclusions: Swelling at the site of vaccination was related to a preexisting immunity. Consequently, farmers and veterinarians are advised to record such swelling per animal; an exclusion of these animals from repeated annual revaccination might be considered. Indeed, prudence is indicated, since "over-vaccination" might become a future problem: Recently very large and persisting swellings were reported in a herd with a repeated annual revaccination.

Milk sampling should include all quarters of the udder! Chronically infected cows need to be identified prior to and controlled after vaccination. Serology is inappropriate to assess immunity in heifers.

Comments: This study was financially supported by the Free State of Bavaria and the Bavarian Joint Founding Scheme for the Control and Eradication of contagious Livestock Diseases.

Herd Health Management: Dairy

P02-002-118

Operating procedures for dairy farms and their servicing veterinary practices- an analysis and optimization of the organization of work processes

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Objectives: In contrast to quality assurance programs in industrial companies a similar model in animal and medical science is only starting to develop. Many farmers and veterinarians lack the awareness of quality assurance programs although they have major responsibility in the food chain. Standard operating procedures (SOP) play a major role in quality assurance programs in industrial production. They provide clear instructions so that fluctuations in product quality are reduced.

The objective of this study was to gain insight into the organization of work processes and analyze the current use of standard operating procedures on German dairy farms.

Materials and Methods: In spring 2015, a questionnaire was handed out to 300 German dairy farmers attending 3 continuing education events. It consisted of 10 questions and 9 statements.

In addition to general farm information (e.g. herd size, annual milk yield, number of employees) the participants were asked regarding the use of SOP (e.g., existence of SOP considering different areas and their documentation). Nine questions evaluated statements referring to how employees handle their tasks, to the documentation of work instructions and a potential interest in SOP available through mobile devices.

Results: Overall 96 questionnaires were returned (32%). On average the farms were milking 170 cows with an annual milk yield of 9.600 kg and a bulk tank somatic cell count of 190.000 cells/ml.

Standard operating procedures were used on 91% of the farms, mostly in areas such as feeding, milking and calf rearing. However, only 51% (41/79) had a written version of their work instructions. On 61% (75/94) of the farms identical tasks were performed differently by different employees and 73% (69/95) of the farmers stated that certain tasks were being performed in a different way than intended by the management. During the creation of operating procedures veterinarians were consulted most frequently as external advisors (68%, 52/77). Nearly all of the surveyed farmers (96%, 89/93) were interested in improving certain areas on their farm and 67% (63/94) of the farmers were interested in using prefabricated SOP and adjusting them to their specific needs.

The majority (69%, 63/91) stated that there is not enough continuing education for the workers and 52% (48/93) did not think that creating SOP was difficult.

Conclusions: There is an obvious discrepancy between the motivation of the farmers to improve the performance on their farm and the expertise in realizing these goals and intentions.

The agricultural sector is observing a trend to larger farms with more animals and a higher percentage of seasonal or migrant work forces. Those farms could benefit from the implementation of high quality SOP as these can help to optimize the management by standardizing the work flow and prevent losses in product quality, work quality and labor efficacy.

Herd Health Management: Dairy

P02-002-119

Predicting and preventing disease in dairy herds using a risk analysis model

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Objectives: The veterinary profession has traditionally been motivated by a model of test and treat where disease outbreaks are recognised, diagnosed and treated and then preventive action taken to prevent recurrence. More effective prevention programmes can be used to predict disease occurring by measuring and monitoring risks, enabling cost effective disease management programmes to be implemented at a herd level to avoid disease outbreaks or limit the impact of any disease within a herd. This study demonstrates the use of a simple web based risk assessment tool to help veterinarians and farmers predict and prevent common infectious diseases.

Materials and Methods: Over 2000 dairy herds have become engaged in various disease management programmes as part of campaigns of awareness and implementation in the United Kingdom. A herd health management tool (myhealthyherd.com) has been used to measure and manage risks pertinent to the particular disease relevant to the herd on these farms as part of their health management.

Risks of disease entry (biosecurity risks) and risk of disease spread (biocontainment risks) were measured by a system of questions, scores and weightings for Bovine Viral Diarrhoea virus (BVDv), Infectious Bovine Rhinotracheitis (IBR), Mycobacterium Avium Paratuberculosis (Johnes Disease, MAP), Bovine Tuberculosis (bTB), Neospora and Bovine Respiratory Disease (BRD). The algorithm in the model then calculated the relative risks of each of these diseases being a problem to the herd, giving veterinarians relevant information on which to base practical preventative advice to minimise the impact of these diseases to the herd.

Results: 2993 herds have completed risk assessments for one or more of the listed diseases. Most showed high levels of risks of specific diseases entering and spreading within the herd, suggesting a high economic impact should the disease exist or be a threat to the herd. Many of the high risk herds were not infected or affected by the particular disease, yet were at high risk, allowing specific advice to be given on biosecurity and vaccination as a preventative rather than a curative. For example, over 70% of herds were at high risk of BVD entering the herd and causing significant impact to the health of the herd, yet less than 10% were actively infected with many of the uninfected herds taking no significant precautions to prevent disease.

There were major geographical differences, particularly related to risks such as stock densities and cattle movements, making some areas much higher risk to disease than others. For example, in some areas, over 76% of herds introduced cattle into their herds without any form of quarantine, and over 50% had cattle drinking from water courses that has passed through other cattle farms.

Conclusions: Many dairy farms are at high risk of infectious disease entering and spreading within their herds, causing significant impact on the health of the herd. Traditionally, many farmers and veterinarians wait for disease to become established and then take action to control and eradicate the disease. This study demonstrates that disease risks can be used to predict and prevent many common infectious diseases entering and spreading in herds thus allowing practical and effective measures to be taken to prevent diseases before they become established.

Herd Health Management: Dairy

P02-002-120

Dairy data quality, much more than just missing data.

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Objectives: Data stored in dairy herd management software could be used by veterinarians for herd health management. Data ought to be of high quality in order to be useful. Analysing data of poor quality will result in erroneous management decisions. For most researchers and veterinarians data quality equals missing data. Data quality however, is acknowledged to be a multidimensional concept. For instance, the recording of milk production data after a dry off event is considered to be a relational constrain violation and has nothing to do with missing data. We aimed to raise awareness regarding data quality being more than a complete dataset.

Materials and Methods: For all herds connected to dairy data warehouse, the database of the herd management software was extracted via an automated tool. The automated extraction tool and procedure are developed and owned by the company Dairy Data Warehouse (DDW) ©. All animals, active and historic, male and female, from the extracted dataset were queried for the following data anomalies: the existence of events prior to the date of birth; the recording of events with a date in the future; the registration of a male animal producing milk; the recording of multiple death events for the same animal and the recording of a negative milk production amount. Errors were logged on animal level and loaded into an excel file. If the same data error occurred multiple times in the same animal, it was only counted for as one.

Results: In total 1,235,279 animals from 333 different dairy herds were extracted. All 5 anomalies were detected. Three percent or 37,264 animals had anomalies in their data. In total 40,822 anomaly cases in 205 different herds were detected. With 61.1%, the error, events prior to the birthdate was the most registered. Negative milk production was the second most important data fault, with 37.2%. In 1.1% of the anomalies an event happened in the future. Male animals producing milk and the recording of multiple death events happened respectively in 0.4% and 0.3% of the recorded cases. The median herd data anomaly rate was 0.1%, defined as the number of animals within a herd with a data anomaly divided by the total number of animals included in the herd database. The maximum herd data anomaly rate was 86.5%. The minimum herd data anomaly rate was 0%.

Conclusions: None of the queried data errors are linked to missing data. Compared to literature, only a low percentage of animals had data errors. This can be explained by the low amount of tested data errors. When more anomalies will be defined and queried, the percentage of animals with a data anomaly will probably increase. All data from animals with events recorded prior to the birthday, is compromised. Hence, the data cannot be used for herd health management. A clear difference in herd data anomaly rate between herds is observed. The reason whether the latter is related to the used software or explicit herd management factors has to be investigated.

Herd Health Management: Dairy

P02-002-121

Link between the histology and endocrine functioning of the pancreas in overconditioned dairy cows at the end of pregnancy

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Objectives: Overconditioning in dairy cows, like obesity in humans, appears to be an important risk factor to develop a wide variety of health problems in the periparturient period. Since the endocrine pancreas plays a central role in the metabolic adaptations at the start of lactation, maladaptations that go along with overconditioning may play a pivotal role in the occurrence of major health disorders. The objective of the present study was to determine the effects of overconditioning at the end of pregnancy on the histology of the pancreas and the functioning of the pancreatic β cells in dairy cattle.

Materials and Methods: Ten pregnant Holstein Friesian cows were selected based on body condition score (BCS) and divided in 2 groups: normally- (≤ 3.5) and overconditioned (> 3.5). During dry period, animals were weekly monitored for BCS and back fat thickness (BFT), and blood samples were taken to determine the non-esterified fatty acid (NEFA) concentration. At an average gestation period of 260 days, cows underwent an intravenous glucose tolerance test (IVGTT) to measure the insulin secretory capacity of the pancreas. A glucose bolus was administered IV and blood samples were taken on different time points to determine glucose and insulin concentrations. Cows were euthanized on average 8 days after the IVGTT, after which 15 pancreatic tissue samples were taken by systematic random sampling. Using a standard protocol, hematoxylin and eosin (HE) stained sections were prepared from the tissue samples. Based on data obtained from the HE stained sections and the IVGTT, macroscopic, histological and functional parameters of the pancreas were determined. Data were statistically analyzed using SAS software. Normality of the data was checked using the Kolmogorov-Smirnov test. To assess the differences between the 2 groups, the PROC ANOVA function and the PROC NPAR1WAY function for resp. normally and not-normally distributed variables were used. Pearson correlation coefficients (r) were calculated between variables representing the pancreatic characteristics and the cow characteristics (BCS, BFT and NEFA level). Significance* and tendency (t) were declared at $P < 0.05$ and $0.05 < P < 0.1$, respectively.

Results: Histological results revealed that overconditioning at the end of pregnancy comes together with fat infiltration in the pancreas and this was accompanied by a relative increase in the amount of endocrine tissue (pancreatic fat%*: 1.29 ± 1.43 ; 6.75 ± 7.11 ; islet%*: 1.28 ± 0.13 ; 1.73 ± 0.15 ; #islets per surface area pancreas (t): 1.74 ± 0.12 ; 2.15 ± 0.37 for the normally- and overconditioned group resp.). In addition, results revealed a positive correlation between the peripheral NEFA concentration and the pancreatic islet percentage ($r = 76^*$) and the number of islets per surface area pancreas ($r = 0.83^*$). The results of the IVGTT revealed that overconditioned animals had a higher insulin secretory capacity of the endocrine pancreas. The peak insulin concentration, the area under the curve (AUC) for insulin for the different time intervals and the acute insulin response to glucose (AIRg) after the administration of the glucose bolus, all tended to be higher in overconditioned cows. In addition, the peak insulin concentration was correlated with the peripheral NEFA concentration ($r = 0.63^*$). No significant effects of the condition or lipid metabolism on the elimination rates of insulin (ER_{ins}) and glucose (ER_{glu}) were found.

Conclusions: It can be concluded that overconditioning at the end of pregnancy in dairy cattle, as obesity in humans, is accompanied by an increase of fat tissue and a concomitant relative increase in endocrine mass in the pancreas, finally leading to an increased activity of the endocrine pancreas. Further research is however warranted to understand the underlying mechanisms with regard to the increased sensitivity towards disease in order to come to more successful preventive strategies and curative treatments.

Herd Health Management: Dairy

P02-002-122

Mastitis detection in modern free-stall barns with conventional parlour milking and automatic milking

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Objectives: Udder health of every individual cow has been well known by the farmers in traditional small tie-stall barns of the Nordic countries. Has this tradition changed after converting to larger farms? Objective of the study was to explore udder health management in modern, large free-stall barns.

Materials and Methods: We studied 82 free-stall dairy farms with an average herd size of 126 cows with conventional parlour milking (50 farms) and automatic milking (32 farms) in Finland in 2012. In an interview, farmers had to select the methods of detecting mastitis used in their farm from a list of 12 choices including a choice "other, describe". Herd health recordings of cow somatic cell count (SCC) and records of quarter milk bacterial analysis were also used to check the reliability of the data inquired. This was a part of a larger study exploring the associations between animal health and grouping of cows.

Results: Eighty-two and 72% of the farms with conventional and automatic milking used test-day cow SCC records and CMT-test to detect mastitis. In conventional milking 94% of the farms checked visual appearance of milk and 64% palpated the udder during milking. In automatic milking 84% of the farms used electrical conductivity and 59% automatic milk colour evaluation. They also monitored milking frequency (69%) and unsuccessful milkings of the cows (56%) in order to detect mastitis. Other choices were used in less than 30% of the farms. Over 90% of all farms used bacterial sampling of a quarter to decide whether to treat or dry it or cull or follow-up the cow. Farmers milking cows automatically used broader spectrum of detection methods in many different combinations.

Conclusions: In conclusion, farmers use a wide range of methods to detect mastitis in modern dairy farms studied. Decisions to treat or not treat a cow with mastitis are based on information of the inflammation status of the udder and on the interpretation of the causative bacteria. These final decisions are made by a veterinarian according to the legislation. Adequate diagnosis, treatment and prognosis are important in order to reduce the consumption of antibiotics and also from animal welfare and economic perspective.

Herd Health Management: Dairy

P02-002-123

Assessment of transition cow management on six UK dairy farms using apps to assess farm management, disease incidence and disease costs

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Objectives: The transition period represents a key time in the dairy cow production cycle, with the outcome having a major impact on health, welfare, production and profitability. The aim was to assess transition cow management and outcomes on six UK dairy farms using novel 'apps' called the Risk Assessment Tool (RAT) and Economic Assessment Tool (EAT) (Elanco Knowledge Solutions ; Elanco Animal Health).

Materials and Methods: Farms received two visits from the author, an experienced dairy vet, at an interval of four months. A structured farm walk was conducted with details entered onto the RAT app, using an iPad. The app requires assessment of management, nutrition and environment to identify key areas where inputs are sub-optimal.

Transition disease levels were assessed from farm records, vet invoices and interviewing the farmer. In addition freshly calved cows were screened for ketosis using milk samples (Keto-test, Elanco Animal Health). The data was used to populate the Economic Assessment Tool to identify management inputs (routine treatments), disease levels and the financial impact of transition period outcomes.

Results: Vet assessment using the RAT identified risk factors for cow health during the far-off, transition and fresh calved periods. The app helped to prioritise the changes needed to achieve the maximum impact. The most common areas for advice were on management and nutrition. Mastitis and metritis had the greatest financial impact, and most herds had underlying ketosis. Identification and recording of the key disease area were used to monitor and to raise awareness on farm. Reassessment four months later found improvement in health and financial performance of £19 per cow calving event, through reduction in disease. The cost of the transition period (investment and disease outcomes) averaged £171 per cow. This was typically £40 in investment (routine treatments and supplements), and the remainder and major cost was disease outcomes.

Conclusions: Transition period management is an area where dairy vets can deliver improved health and profitability. The apps developed provided a structured method for assessing transition period management and outcomes. Many of the areas identified for change involved people and nutrition, and so barriers could be overcome without significant capital expenditure. Change advised proved to be effective in reducing disease and improving the farm financial position.

Comments: Further data will be added showing individual farm outcomes and statistics if abstract accepted.

Herd Health Management: Dairy

P02-002-124

Mycoplasma bovis Seroprevalence in Healthy Dairy Cattle in Turkey

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Objectives: M. bovis in cattle exacerbates the respiratory disease. In 128 BRD outbreaks in France and Germany, M bovis was isolated combined with M. haemolytica and / or other bacteria. This pathogen has been considered a silent one, but increasing the damage in the respiratory tract and promoting relapses of BRD. In Turkey, the role of this pathogen is not completely known. The objective of this study was to determine the sero-prevalence of M. bovis in sera of healthy animals.

Materials and Methods: Blood samples from animals collected from different regions in the seven provinces of Aegean area (Izmir, Manisa, Aydin, Mugla, Usak, Kutahya, and Denizli provinces). Sera samples were taken for routine analysis for Brucellosis monitoring from certified dairy farms. These farms are free from tuberculosis too. Some of these cattle had imported from different countries. A total of 624 blood samples, coming from 21 different farms were analyzed by ELISA test (Bio-X Diagnostics) and results were calculated according to manufacturer's manual.

Results: Eventually 24% of sera were evaluated as positive. Positive results shown different ratios between different farms. These animals belong to the west region provinces of Turkey. All farms were intensive dairy facilities. These results have shown a high frequency of seropositive animals considering that they are healthy and well-fed. These farms could describe "good managing and feeding" farms to rest of other farms. Farmers and breeders prefer to buy these cattle in spite of high prices primarily because of healthy conditions.

Conclusions: M. bovis infections point out high ratio of sera positive results, because of that, these infections must be considered in mastitis and BRD infections. Further studies with PCR in milk or from BRD affected animals would give a better understanding of the distribution of M bovis in Turkey.

Herd Health Management: Dairy

P02-002-125

Differences in neck movements associated with rumination recorded by accelerometers affixed to neckbands in different dairy cattle herds

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Objectives: Our research group succeeded in obtaining wireless recordings of neck movements associated with rumination in dairy cows using a three-dimensional accelerometer attached to a neckband. Rumination behavior in cows is essentially a physiological response associated with digestion and is strongly correlated with feeding and milk production. However, accurately monitoring the temporal characteristics associated with rumination behavior for clinically and herd health assessments in individual herds is difficult. This study compared rumination data, herd characteristics (e.g., milk production) and environment on three different farms.

Materials and Methods: Twenty-four Holstein cows in free-stall barns

on three different farms (A, B and C) in the Kushiro area of Hokkaido were used in this study. Wireless 3-axis accelerometers affixed to commercial neckbands were used to record neck movement data in ruminating cows. These data were automatically transferred and stored on a data server over a local wireless network (WiSUN, IEEE802.15.4g) and the internet. Cow behavior was simultaneously recorded on digital video in order to correlate the obtained acceleration data with cow movements. The sampling frequency of the accelerometers was set to 20 Hz and the length of each stored dataset was 1024 points. In order to compare the differences among farms, the collected data were compiled and analyzed using Excel (Microsoft) and MatLab (MathWorks Inc.). Analysis methods were used autocorrelation and histogram in each direction data, and 3-direction resultant vector and difference of 3-direction resultant vector. The Penn State Particle Separator (PSPS) was used to quantitatively determine the total mixed ration (TMR) in each farm. On each farm, products information and information of oral interview were also collected in experimental cows. The data obtained from the three farms were compared using ANOVA. This study was approved by the Animal Research Committee of the Faculty of Agriculture at Iwate University, Japan. Experiments were conducted according to the Iwate University Guidelines for Animal Experimentation.

Results: The acceleration waveforms obtained during rumination were markedly different among farms A, B and C. After calculating the autocorrelation function, the amplitudes of left-right and up-down movements for cows on farm C were significantly higher than those for cows on the other farms ($p < 0.01$). For cows on farm C, the interval between peaks of head movement has a strong tendency to change periodicity pattern. The granularity of the TMR for each farm was as follows: < 1.7 mm, A: 32.4%, B: 17.3% and C: 18.6%; 1.7 to 8.0 mm, A: 61.8%, B: 43.1% and C: 39.9%; 8.0 to 19.0 mm, A: 5.8%, B: 30.4% and C: 36.5%; > 19.0 mm, A: 0.0%, B: 9.2% and C: 5.0%. The average milk yield was 25.6 ± 1.6 kg/day on farm A, 24.7 ± 2.4 kg/day on farm B, and 31.1 ± 1.6 kg/day on farm C. The sufficiency ratios of the cattle beds on each farm were 71% on farm A, 76% on farm B, and 106% on farm C ($p < 0.05$).

Conclusions: Rumination is a physiological reflex with chewing. However, no studies have demonstrated that the movements associated with chewing are affected and related by rumination. In this study, differences in rumination behavior (inferred based on accelerometer and video data) were observed among cows on different farms. It is considered that these differences were due to the granularity of the TMR contents and environmental factors between farms. Furthermore, the periodic and greater amplitude of acceleration during rumination may also related with farmer's productivity and environmentally appropriate.

Herd Health Management: Dairy

P02-002-126

Serum protein profile of Holstein cows during the transition period

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Objectives: The transition period is characterized by metabolic adaptations in order to supply the energy demands at the end of pregnancy. In this context, the use of biomarkers such as serum proteins promotes a multifunctional profile and evaluation of inflammatory and

immunological condition of cows during this period. The aim of this study was to evaluate longitudinally the serum protein profile during the transition period in Holstein cows bred in Brazil.

Materials and Methods: Thirteen pregnant cows were evaluated, from second to fourth lactation. Data of the occurrence of disease during the study period were obtained by consulting the farm records. At the time of sample collection, it was also evaluated the body condition score (BCS). Blood samples ($n=78$) were taken weekly, from the second week before calving (-2) to the third week in milk (+3) including calving (0), for determination of serum protein profile, consisted by determination of total serum protein (TSP); negative acute phase protein (albumin - ALB and transferrin - TF); positive acute phase protein (haptoglobin - HP; acid glycoprotein - AG and ceruloplasmin - CP), and also the heavy and the light chain of IgG and IgA, by SDS-PAGE electrophoresis.

Results: PT values decreased gradually ($P=0.000$) from 6.4 g/dL in week -2, to 6.2 g/dL at calving (0), increasing in subsequent moments ($+3=7.3$ g/dL). The concentrations of the heavy chain ($-2=919.4$; $-1=1074.5$ mg/dL) and the light chain of IgG ($-2=393.9$; $-1=466.7$ mg/dL) were lower before calving compared to postpartum ($+1=1,283.3$; $+2=1,374.2$ and $+3=1,630.3$ for the heavy chain and $+1=463.4$; $+2=573.7$ and $+3=651.8$ for the light chain). For IgA the values decreased ($P=0.001$), from 51.9 mg/dL (-2) to 34.0 mg/dL (+1), and increased in +2 (45.4 g/dL) and +3 (62.6 g/dL). The values of HP and CP increased ($P=0.000$) from -2 (HP=16.6 mg/dL; CP=8.6 mg/dL) to +3 (HP=60.9 mg/dL; CP=127.1 mg/dl). ALB showed slight variations during the transition period ($P=0.000$), while the TF ($P=0.101$) and AG ($P=0.105$) were stable. For the BCS it was observed difference ($P=0.003$) between -2 (ECC=4.0) and +1 (ECC=3.0). It was reported the occurrence of dystocia (4/13), retained placenta (1/13) and hypocalcemia (1/13) on the day of calving (0) and uterine infections (5/13) and ketosis (1/13) occurred in the postpartum. The lowest values of total proteins and immunoglobulins (Igs) obtained before calving is related to colostrum development or, in the case of Igs, to reduction in the amount and function of B lymphocytes, which may cause immunosuppression. Increasing concentrations of positive acute phase proteins around calving can be explained by the increase of their synthesis after liver stimulation induced by pro-inflammatory cytokines during calving. In contrast, negative acute phase proteins were inhibited.

Conclusions: In conclusion, these factors linked to the high BCS before calving and abrupt lost weight after calving probably might have caused accentuated metabolic and immunological changes during the transition period, increasing the susceptibility of cows to bacterial infections from the first week after calving.

Herd Health Management: Dairy

P02-002-127

Subclinical ketosis testing as a part of herd health management

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Objectives: Subclinical ketosis reduces milk yield and impairs fertility and immunity of dairy cows. Objectives of this study are to 1) test suitable subclinical ketosis tests and different testing protocols as tools for herd health management, to 2) evaluate the value of routine tests of milk acetone and BHB for farmers and veterinarians, to 3) explore the incidence of subclinical ketosis in Finnish dairy herds, and to 4) compare the ketosis risks between Holstein and Ayrshire cows.

Materials and Methods: In Finland, 6000 dairy herds DHI samples were checked for milk acetone and BHB with FOSS MilkoScan™ FT+/6000 for three months, during October to December 2015. Among those herds, 20 herds were selected for additional weekly milk sampling, and two herds for additional clinical examination and blood sampling of the cows that were at the greatest risk for subclinical and clinical ketosis.

Two high-producing dairy farms were visited once a month for three months. During the farm visit, dairy cows between 5–50 days postpartum were examined. Together with full clinical examination, urine, milk and blood samples were taken for analysis. Cow-side tools used were Keto-test BHB (β-hydroxybutyrate) dipsticks, Ketocheck powder and BHB-check analyzer. Serum samples were used to analyse non esterified fatty acids, BHB, cholesterol and triglycerides.

The incidence and prevalence of subclinical ketosis will be measured with the different tests, and sampling frequencies used in this study. The association between subclinical ketosis and breed of the cow will be modelled with a logistic mixed model, with herd as a random effect. Herd and cow data are gathered Finnish national dairy herd recording database, operated by ProAgria Agricultural Data Processing Centre.

Results: The results will be available by the time of the congress, because the cow-side and laboratory analyses will continue until the end of December 2015. We will present a way to utilize subclinical ketosis testing as a part of herd health management planning routines of the farmer and his veterinarian. The value of the different tests and sampling routines explored will be compared and described. The incidence on subclinical ketosis in our study population will be presented, including the comparison of the incidence risks of Holstein and Ayrshire cows.

Conclusions: Testing the cows for subclinical ketosis is useful in evaluating the feeding, housing and management of high producing dairy cows. More in depth protocols for comprehensive herd health management are needed, and should be further developed.

Herd Health Management: Dairy

P02-002-129

Prevalence of subclinical ketosis monitored by a semiquantitative cow-side milk beta-hydroxy-butyrate test in Hungarian dairy herds

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Objectives: The objective of the study was to examine the prevalence of subclinical ketosis (SK) manifested in ketolactia in Hungarian large-scale dairy herds. Further aim was to analyse the relationship between some cow-related and management factors, the different clinical diseases and the SK.

Materials and Methods: 491 Holstein Friesian dairy cows in <35 days in milking kept in eight different large scale dairy farms in Hungary were involved in the study. The examinations regarding the concentration of beta-hydroxy-butyrate (BHB) of the milk were conducted on-site, from milk samples, using a colorimetric semi-quantitative test (Keto-Test™). Data of each of the cow- and management related factors (number of lactations, previous lactation production, number of milked cows, housing technology) and clinical diseases (retained placenta, metritis,

mastitis, abomasum displacement, diarrhoea, lameness) were recorded. The relationship between the different variables were analysed by generalized estimating equations statistical model.

Results: Different severity of SK (≥100 μMol/L milk BHB) was diagnosed in 38% of the animals examined. SK prevalence showed a significant variance in the eight examined dairy herds (25-57%). The probability of SK with increasing number of lactations showed a non-significantly rising tendency. The milk production of the previous lactation and the size of the herd did not show significant relationship with the odds of SK. In the case of cows suffering from various diseases the concurrent presence of SK was diagnosed with high prevalence (>50% on average).

Conclusions: Although the prevalence of SK is remarkably high in Hungarian dairy herds, a significant variance was detected in the eight examined farms. The parallel onset of clinical diseases and SK was found in a high rate. The implementation of the Keto-Test™ colorimetric semiquantitative milk BHB cow-side test is a useful tool for monitoring ketosis in dairy herds. The diagnosis of SK may indicate the higher risk of other clinical diseases in the periparturient period in dairy cows.

Comments: The authors express thanks to Elanco Animal Health and Vet-Produkt Ltd. for the material and logistic support of this study.

Herd Health Management: Dairy

P02-002-130

Understanding the irrational dairy farmer - a study into farmers' decision making

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Objectives: In WBC Chile 2010 we presented a set of research-based recommendations to bridge the gap between science and practice in dairy herd health management.¹ Now, with cow-mortality as an example, we offer insight into our approach method from a larger field study.

Materials and Methods: We described the theory and methods in 2011². Lately, we have found additional inspiration in the concept of Motivational Interviewing³ and the paper by van der Leek.⁴

We studied 12 very different dairy herds with cow-mortality risk exceeding 10 % per lactation. However, the true study-objective was the interaction between farmer, veterinarian and nutritionist - not cow-mortality per se. We acted as neutral observers to identify and prioritize possible causes of high cow-mortality.

First, participants were presented with a list of possible general causes of cow-mortality at herd level (e.g. lameness, farming skills, poor advisor, stable, staff, lack of faith in own abilities). Second, we asked each participant to identify and rank the three causes of high cow-mortality in this particular herd they judged most important. Third, participants discussed their choices to reach a shared understanding. Next, we presented an estimate of the financial gain related to a 50 percent reduction of cow-mortality. Finally, the participants attempted to agree on a herd-specific action plan to reduce cow-mortality.

We studied the choices and arguments made by the participants, individually and in the group, and compared them to our own interpretation of the situation. Also, we estimated the participants' combined motivation to succeed, their professional knowledge and their level commitment to implement the action plans. Further, all farms were visited in the middle and at the end of the study to learn from the farmer's perception of failure or success.

Results: Obviously, results must be interpreted and understood within the context of each farm. Across herds there was no obvious effect on cow-mortality and no obvious relation between estimated financial gain and observed motivation or level of commitment. Three examples follow as illustration:

Example #1: Participants shared the perception that high cow-mortality was related to lameness in the herd. Consequently, the farmer changed floor type. Now, participants are waiting for cow-mortality to decrease. We would have preferred a validation of the evidence before such an expensive intervention. We still follow this farm because we believe that the participants' poor understanding of causality lead them to un-justified conclusions.

Conclusions: We conclude, that 1) farm-level professional knowledge often was insufficient; 2) herd health advisors must learn how to motivate change; 3) level of commitment was unclear; 4) profit is not (always) a driver. If veterinarians want to qualify themselves to be trustworthy dairy herd health management consultants in the future, these issues must be addressed. Consequently, farmers are not to be perceived as irrational if they do not follow the advice presented by a herd health advisor. Actually, some skepticism towards recommendations seems to be justified.

Comments: ¹ Kristensen & Jakobsen, EL, EB, 2010. (E-)Valuation of Dairy Herd Health Management. Abstract from WBC Chile, pp. 53-64

² Kristensen & Jakobsen, EL, EB, 2011. Challenging the myth of the irrational dairy farmer; understanding decision-making related to herd health. NZVJ, 59; 1-7

³ Miller & Rose, WR, G, 2009. Toward a theory of motivational interviewing, American Psychologist, 64; 527-537

⁴ van der Leek, M. Beyond traditional dairy veterinary services: 'It's not just about the cows!'. JSAVA, 86; doi: 10.4102/jsava.v86i1.1221

received a tube with internal sealing the base of bismuth subnitrate via intramammary. We evaluated the serum fibrinogen, albumin, total protein, beta hydroxybutyrate, nonesterified fatty acids, insulin, glyco-
 cose, total cholesterol, highdensity lipoprotein (HDL), lowdensity lipoprotein (LDL) and triglycerides levels, the sample were collected at six moments, namely, M1, at the time of drying (60 days before delivery), M2 on the day of delivery, M3 3 days post calving, M4 seven days, M5 10 days, M6 21 days and M7 with 30 days post partum.

Results: The serum fibrinogen levels, an indicator of inflammatory disease, were correlated with HDL ($r = 0.21$; $P = 0.03$) and total protein ($r = 0.24$; $P = 0.015$) levels. The serum albumin levels were related to tryglycerides ($r = 0.25$; $P = 0.012$) and cholesterol ($r = 0.32$; $P = 0.001$) levels. The serum total protein levels were associated with an increase of insulin ($r = 0.25$; $P = 0.017$); triglycerides ($r = 0.36$; $P = 0.0002$), total cholesterol ($r = 0.22$; $P = 0.03$), LDL ($r = 0.28$; $P = 0.0046$) and HDL ($r = 0.34$; $P = 0.0005$) levels. Furthermore, the insulin levels were related to glyco-
 cose ($r = 0.32$; $P = 0.002$), tryglycerides ($r = 0.29$; $P = 0.005$), and BHB ($r = 0.22$; $P = 0.04$) levels. The NEFA levels were correlated with BHB ($r = 0.62$; $P < 0.0001$) and LDL ($r = 0.27$; $P = 0.007$) levels. The tryglycerides levels were associated with BHB ($r = 0.27$; $P = 0.008$) and glyco-
 cose ($r = 0.36$; $P = 0.0002$) levels. Finally, the cholesterol levels were correlated with HDL fraction cholesterol ($r = 0.47$; $P < 0.0001$) levels.

Conclusions: We conclude that, metabolic changes associated with energy imbalance and fat mobilization was related to alterations in protein metabolism, and probably with immune dysfunction, and susceptibility to diseases.

Comments: In face of, we believe that this approach has the potential to provide new diagnostic and decision support tools to improve animal health, whilst simultaneously maintaining optimal production and efficiency.

Herd Health Management: Dairy

P02-002-131

Relationship among inflammatory and metabolic measures during the immediately postpartum period of Holstein dairy cows

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Objectives: Increased disease rates are commonly reported among highyielding dairy cows during the immediately postpartum period. Thus, the present study aimed to evaluated the relationship among some inflammatory indicators and metabolic measures during the immediately postpartum period in Holstein dairy cows.

Materials and Methods: Were used 34 cows reared in semi-intensive system, with average production of about 19 liters of milk per cow. These were allocated evenly into three groups that received different treatments at the time of drying. The control group (Group 1), with 13 animals that did not receive drying treatment. The antimicrobial intramamário Group (Group 2), with 10 animals that were treated after the last milking with a tube on each breast with room for antimicrobial dry cow Cefalônio anhydrous basis (0, 25 g of anhydrous cefalônio) and the inner sealant Group (Group 3), with 11 animals that

IBR

P02-002-226

Bovine herpesvirus 5 (BoHV5) subtype b is unexpectedly a recombinant virus between BoHV5 and BoHV1, the agent of infectious bovine rhinotracheitis

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Objectives: Bovine herpesvirus 5 (BoHV5) is the agent of bovine encephalitis occurring especially in South America, together with BoHV1, responsible for infectious bovine rhinotracheitis (IBR). Among the 3 BoHV5 subtypes, subtype 'b' was until now only detected in Argentina, and seems to be less prevalent. Both BoHV1 and BoHV5 belong to ruminant alphaherpesviruses and share a high genetic similarity. Under experimental conditions, we obtained interspecies recombinants between the involved viruses, BoHV1 and BoHV5. We report here for the first time the identification of homologous recombination between BoHV1 and BoHV5 in field virus isolates.

Materials and Methods: Two fragments of the three BoHV5b isolates were amplified by PCR, then sequenced and a phylogenetic inference was performed using the MEGA version 4 software package. Tree topology was constructed by maximum composite likelihood method with the Tamura and Nei model (1000 replicate bootstrap values). After we amplified by PCR the complete UL27 gene of three BoHV5b isolates, sequenced and genetic recombination was analysed using a sliding-window genetic diversity plot (Simplot software version 3.5.1 available at <http://sray.med.som.jhmi.edu/SCROftware>) and the Recombinant Detection Program (RDP), version 3, available at <http://darwin.uvigo.es/rdp/rdp.html>).

Results: We showed by phylogenetic analyses that these three BoHV5b isolates clustered differently with BoHV1 and BoHV5 depending on the genes encoding glycoprotein B (gB) or D (gD) suggesting that these viruses could be recombinants between BoHV1 and BoHV5. We detected two recombination breakpoints located in the long unit (UL) genomic region within the UL27 gene open reading frame. Based on the BoHV5 UL27 gene (GenBank accession number YP003662497.1) one recombination breakpoint extended from the nucleotide (nt) 475 to nt 491 and the other one from 2749 nt to 2761 nt giving a recombination fragment of 2286 base pairs showing 100% nucleotidic identity with the BoHV1.2b subtype genome. Other genomic regions, encompassing five gene fragments from UL and two from the short unit (US) region, were also analyzed by PCR and sequencing, resulting in 100% nucleotide identity with the subtype BoHV5a genome.

Conclusions: Homologous recombination between two related ruminant alphaherpesviruses was shown in natural field conditions. We found three recombinant field isolates between BoHV1 and BoHV5, previously known as BoHV5b subtype. The existence of natural recombination and also the co-circulation of different ruminant alphaherpesviruses increase the complexity to carry on programs of control and eradication of IBR. Especially their impact on BoHV1 differential diagnosis methods and the risk to escape vaccine-induced immunity depending on the degree of antigenic variation caused by recombination should now be taken into account.

IBR

P02-002-227

A new diagnostic approach to serological surveillance of IBR marker vaccinated dairy herds in Cuneo province: a field study

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Objectives: In Italy, control measures have been implemented in order to reduce the economic losses caused by Bovine Herpesvirus 1 infection. Since 2004 an eradication programme is in place in Piedmont region. In order to contain the costs of IBR surveillance, the use of bulk milk, has to be considered as a possible diagnostic solution. The recent introduction of an indirect gE ELISA using milk IgG sequential precipitation (Eradikit® BMSK Plus, In3diagnostic) seems a promising tool for IBR surveillance of vaccinated herds. The method was applied to pooled milk samples in dairy herds and compared with individual serum assay over a 12 months period.

Materials and Methods: Three hundred dairy farms of Cuneo province, comprising 28000 lactating cows, were enrolled in this study. Individual serum samples were collected and tested by whole virus and gE blocking ELISAs, and sanitary status updated as follows: 158 gE negative and 142 positive herds. Individual milk samples were monthly collected by breeder association according to periodical milk parameter determination and were used to make milk pools (n=1365 pools in two sessions) of up to 40 lactating cows (median 34, IQR 30-37). Therefore, considering the outcomes of the official test in serum samples, the within-pooled milk prevalence was calculated and the field sensitivity of the method was assessed. Pool samples were processed for IgG concentration and indirect ELISA procedure, according to manufacturer protocol. The results of the first two sessions of analyses are reported below.

Results: All pools from gE negative farms scored negative to Eradikit® BMSK-plus (specificity: 100%, 95%CI: 97.7%>100%). Among positive farms, all but 2 (n=69) having IBR prevalence of lactating cows greater than 2.5% scored positive, while dairy herds having IBR prevalence lower than 2.5% scored negative in all pools. In the latter farms gE positive serum samples were mainly attributable to old vaccinated animals. In one occasion a previously classified gE negative farm turned positive during the serological annual control. Pooled milk test, carried out in two occasions before the annual control, readily detect viral circulation.

Conclusions: Field specificity and sensitivity of Eradikit® BMSK-plus seems adequate for surveillance of gE negative herds and for detection of viral circulation at prevalence level greater than 2,5%. However, during the late stage of eradication, the proposed method would require reduced pool size to detect a weak positive (old vaccinated) sample.

IBR

P02-002-228

Patterns of exposure to bovine herpesvirus-1 and -4 in Irish cattle

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Objectives: Herpesvirus infections cause a range of clinical syndromes in cattle – respiratory, reproductive, systemic and nervous. Although bovine herpesvirus 1 (BoHV-1) is the most prominent due to semen collection centre controls and eradication programmes in several European countries, bovine herpesvirus 4 (BoHV-4) shares many of the former's features in terms of life cycle and clinical signs. This study for the first time explores the patterns of exposure to BoHV-1 and BoHV-4 in Irish cattle and examines and compares the associations with individual animal and herd level factors.

Materials and Methods: A formally constructed random survey (1,589 sera from 318 herds) was conducted in early 2013 within the last full national testing round for Brucellosis. Samples were tested by ELISA for antibodies to BoHV-1 and BoHV-4 using blocking (IBR gB Ab, IBR gE Ab; IDEXX Laboratories, US) and indirect (BHV-4 Ab BioK 312; BioX diagnostics, Belgium) ELISAs respectively, according to the manufacturers' instructions. Contemporaneous descriptive and epidemiological data at animal and herd levels at the time of sampling were obtained from the Animal Health Computer System (AHCS) of the Department of Agriculture, Food and the Marine (DAFM). Both descriptive and regression analyses were performed. An animal status of BoHV-1-infected was assigned if testing positive by both IBR gB AND IBR gE ELISAs, while a status of BoHV-1-vaccinated was assigned to animals testing IBR gB ELISA positive AND IBR gE negative.

Results: Individual animal antibody prevalence rates were 31%, 14% and 44% for BoHV-1 infected, BoHV-1 vaccinated and BoHV-4 infected respectively. The mean within-herd prevalence for BoHV-1-infection, excluding negative herds, was 12.3% while the mean within-herd prevalence for BoHV-1 vaccinated, excluding negative herds, was 6.3%. The mean within-herd prevalence for BoHV-4 infection, excluding negative herds, was 14.0%. For both BoHV-1 infection and BoHV-1 vaccination, the distribution of within-herd prevalence levels was biased to the left, so that incrementally a smaller proportion of herds show higher prevalence rates. However this pattern was not observed in BoHV-4-infected herds, where 100% of samples tested positive in 17% of herds. The mean individual prevalence levels for BoHV-1 infection was similar across Dairy, Beef, Suckler and Other herd types (as listed on AHCS) at 32.3% ,but much higher in Dairy herds for both BoHV-1 vaccination and BoHV-4 infection at 21% and 73%, respectively. The strongest breed association was a high seropositivity (78%) to BoHV-4 in Friesians. Individual animal age had a clear positive association with antibody prevalence to both BoHV-1 and BoHV-4 infections but not BoHV-1 vaccination. If an animal was still in its herd of birth this was strongly associated ($p < 0.0001$) with a negative BoHV-1 infection status, but no association was detected with BoHV-1 vaccination or BHV-4 infection.

Conclusions: BoHV-1 and BoHV-4 infections are frequent and widespread throughout Ireland. The two viruses appear to have independent distributions and patterns of exposure which may reflect the long-standing awareness of and available active control measures for BoHV-1 in contrast to BoHV-4. In particular, the striking association of BoHV-4 infection with dairy herds and Friesian-breed cattle is worthy of further investigation and research.

IBR

P02-002-229

Rapid serological detection of Bovine herpesvirus-1 using nanosensors.

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Objectives: Bovine herpesvirus-1 (BoHV-1), causative agent of infectious bovine rhinotracheitis (IBR) is an important respiratory pathogen of cattle internationally. IBR spreads rapidly through a herd once introduced, and early detection is critical in preventing transmission to herd cohorts. This is especially important in semen collection stations and cattle feedlots where losses due to the spread of BoHV-1 can be considerable. The objective of this project was to develop a nanosensor-based prototype to allow rapid serological detection of BoHV-1 in bovine serum samples.

Materials and Methods: A fully integrated electrochemical-based nanowire platform was fabricated on a silicon wafer using electron-beam lithography. The nanowires were functionalised using an electrodeposited polymer in order to form the attachment chemistry. The commercially sourced whole virus BoHV-1 (APHA Scientific, UK) was covalently attached to the on-chip gold nanowires using a solution of N-ethyl-N-(dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS). Ethanolamine was deposited as on the nanowire after the protein immobilisation as a blocking step to fill remaining unbound active sites. Ferrocene monocarboxylic acid (FcCOOH) in phosphate buffered saline (PBS) were purchased from Sigma-Aldrich and used as a redox probe to undertake the cyclic voltammetry and electrochemical impedance analysis. Archived BoHV-1 positive and negative bovine serum samples were provided by Teagasc (Moorepark, Ireland). Detection of BoHV-1 antibodies (Ab) in serum was undertaken using electrochemical detection methods i.e. cyclic voltammetry (CV) and faradaic electrochemical impedance spectroscopy (EIS) were performed to track antibody-antigen (Ab-Ag) binding on the nanowire surface.

Results: The CV and EIS response obtained after each step in the nanowire functionalization showed an increase in the impedance nyquist semicircle and a decrease in the current intensity for the CV plots. This is the expected response as the modification step is limiting the charge transfer from the FcCOOH at the nanowire surface. Detection of the BoHV-1 antibody was first undertaken in buffer solution, followed by detection in 1% bovine sera. The sensor showed a significant increase in nyquist plot after emersion in the 1 µg/ml Ab/buffer solution for 20 minutes, which indicates that the antibody successfully bound to the immobilised virus on-chip. Furthermore, the sensor exhibited the same behaviour in the presence of BoHV-1 positive bovine serum demonstrating that the target antibody present in infected bovine serum is captured by the virus probe. Negative (uninfected) bovine serum samples caused minimal electrochemical change. This highlights that the sensor is specific for serological detection of BoHV-1 and there is no interference from non-specific binding. With the development of a microfluidic system the response time and sensitivity may be improved. Additionally, as there are six independent nanowires on each sensor chip, it is proposed to develop a multiplexed device which would allow simultaneous detection of both anti-gE and anti-gB antibodies using specific viral antigens, rather than whole virus.

Conclusions: A lab-based biosensor prototype has been successfully developed which allows rapid detection of anti-BoHV-1 antibodies in bovine serum. The ultimate aim is to deploy this nanosensor to a farm environment, which will allow real-time identification of BoHV-1 seropositive individuals. The rapidity with which results can be generated

will allow potentially infectious animals to be isolated in a timely manner, thereby reducing further herd transmission of the virus.

IBR

P02-002-230

Vaccination with a modified-live followed by an inactivated IBR marker vaccine prevents abortion in cattle following challenge with abortogenic BoHV-1

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Objectives: Modified-live and inactivated gE deletion marker vaccines against bovine herpesvirus 1 (BoHV-1) are widely used. Efficacy of a combined protocol with two vaccines containing BoHV-1 gE negative strain Difivac has been shown against respiratory disease but not yet against abortion. Here, the results from a masked, randomised, negatively controlled study are reported where pregnant heifers were challenged with abortogenic BoHV-1 after a single dose priming with a modified-live marker BoHV-1 vaccine (Rispoval® IBR Marker Vivum, Zoetis) and a single dose booster with an inactivated marker BoHV-1 vaccine (Rispoval® IBR Marker Inactivated, Zoetis).

Materials and Methods: Forty-four (44) Holstein heifers, aged between 14 and 16 month and without antibodies to BoHV-1, were enrolled into the study and randomly allocated to one of two treatment groups. The vaccinated group was first vaccinated with a modified-live vaccine (Rispoval® IBR Marker Vivum, Zoetis) followed by a second vaccination six months later of the now pregnant heifers with an inactivated vaccine (Rispoval® IBR Marker Inactivated, Zoetis). In the non-vaccinated control group, heifers received two mock-vaccinations with water for injection at the same time-points. Approximately three months after the final vaccination, between 203 and 204 days of gestation, 12 pregnant heifers of each group were randomly selected and challenged intravenously with the abortogenic BoHV-1 strain Cooper. The impact of vaccination on serology, clinical parameters, abortion rate and off-spring viability was compared to 12 non-vaccinated control heifers. Tissue samples of aborted fetuses or stillborn calves were collected for serology, virus isolation, immunohistochemistry and histopathology. All sample analysis was conducted by personnel masked to the treatment group allocation of individual animals.

Results: Following experimental challenge, nine out of 12 control heifers (75%) aborted. Histopathology in lung, liver and kidney and BoHV-1-specific immunohistochemistry on lung, liver and kidney samples confirmed that all nine abortions were attributable to BoHV-1 infection. Ten out of 12 vaccinated heifers gave birth to normal healthy calves and the remaining two vaccinated heifers had a stillbirth. Sera from nine healthy calves were seronegative in the BoHV-1 serum-neutralisation test (pre-colostral blood sample); one healthy calf had a titre of 48. Sera collected from the two stillborn calves were negative in the BoHV-1 serum-neutralisation test, and no evidence for BoHV-1 infection could be found at histopathological investigations of samples from the calves' lungs, livers and kidneys. BoHV-1-specific immunohistochemistry was negative and BoHV-1 isolation on liver samples was also negative. Therefore, these two stillbirths were not attributed to BoHV-1 infection. There was a significant difference ($p=0.0158$) between treatment groups in the number of abortions and stillbirth attributable to BoHV-1 infection using Fisher's Exact Test.

Conclusions: This study demonstrates that administration of the modified-live BoHV-1 marker vaccine used in this study (Rispoval® IBR Marker Vivum, Zoetis) followed six months later by the inactivated BoHV-1 marker vaccine (Rispoval® IBR Marker Inactivated, Zoetis) induces protective immunity against infection with abortogenic BoHV-1, as demonstrated by an absence of abortions and stillbirths due to BoHV-1 and an absence of in utero infection of the foetus in 11 of 12 pregnancies.

IBR

P02-002-231

Free from Bovine Herpesvirus 1 (BoHV1) is not the end of the story

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Objectives: Since October 2011 the federal state of Bavaria is listed as an article 10-region according to directive 64/432/EEC. Serological cross-reactivity due to BoHV2 infection interferes with BoHV1-tests. A modified gE-ELISA (Idexx, Ludwigsburg, cut-off 0.95) solved the problem at the level of blood testing (Böttcher et al., 2012), but the problem remained unsolved at the level of bulk-milk (BM)-surveillance.

An indirect gE-ELISA for BM-testing (Eradikit BoHV1-gE, bulk milk surveillance kit, In3diagnostics, Turino (IT)) was compared with the routine procedure of BM-surveillance.

Materials and Methods: Surveillance is currently performed by BM-testing with Trachitest (Idexx). Non-negative results are confirmed by a second manually collected BM (Trachitest) and/or blood samples. Blood samples are tested with Trachitest/gB-ELISA (Idexx) and in case of less than four gB-positives per herd the modified gE-ELISA is applied.

The protocol of Eradikit for IgG-concentration was compared with ammonium sulfate precipitation (ASP) for IgG1 (mg/ml) and Eradikit results.

Eradikit protocol was modified to increase the sensitivity: The pellet after ASP was dissolved in sample diluent and tested without any further dilution. The positive control was diluted 1/8 (instead of 1/2) and sample incubation was performed overnight at 4°C (instead of 1 hour at room temperature). A preliminary cut-off at 50% was implemented.

Eradikit was analyzed with 6 positive milk samples supplied by the manufacturer and 17 individual milk samples from two non-vaccinated and BoHV1-infected dairy cow herds, kindly supplied by Antonio Barberio and Stefano Nardelli (Istituto Zooprofilattico, Padova (IT)). Additionally, 20 BMs from a BoHV2-affected (18 non-negative in Trachitest) and 22 BMs from a non-affected Bavarian region (negative in Trachitest) were included. BoHV1-positive individual milk samples were diluted 1/25 and 1/50 in negative milk and subsequently processed as BM in Trachitest and Eradikit.

Finally, BMs from 387 herds with initial non-negative results in Trachitest, which were not confirmed by the routine procedure, and 8 BMs from herds with confirmed BoHV1 were parallel tested with Eradikit.

Results: ASP was simple, cheap, robust and gave better results i.e. higher IgG-concentration, higher detection rate of positive samples and lower background. Additionally, the re-suspended volume allowed testing of duplicates or even retesting.

50% and 70% of the spiked BoHV1-positive milk samples scored positive at a dilution 1/50 and 1/25, respectively. In contrast, only one sample of the BoHV2-negative region scored positive.

Implementation of Eradikit at the level of the first BM reduced the number of suspicious herds from 387 (100%) to 14 (3.6%). In 2015 an outbreak of BoHV1 occurred, BMs from 8 affected herds tested positive in Eradikit with values (OD%) $\geq 150\%$.

Conclusions: Eradikit is a suitable tool to improve BM-surveillance in article-10-regions; it speeds up the surveillance and reduces the costs. However, the current paradigm of BoHV1-surveillance needs to be changed. Currently, it relies on detection of one weak-positive milk sample in a pool of 50 in order to detect a single latently infected cow in a herd. This is very useful in regions with eradication programs (i.e. Article 9), but surveillance in article-10-regions should rather focus on rapid detection of farms with re-infection which is associated with virus spread, strong ELISA-reactivity and high seroprevalence.

Comments: This study was financially supported by the Free State of Bavaria and the Bavarian Joint Founding Scheme for the Control and Eradication of contagious Livestock Diseases.

IBR

P02-002-232

IBRV Reproductive Loss- Vaccine or Field Virus Using Single Nucleotide Polymorphisms (SNPs) to Sort out Vaccine-Induced Reproductive Disease

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Objectives: Modified live virus (MLV) bovine herpesvirus 1 vaccines have been a major aid to the prevention of reproductive disease in cattle. However their use (or misuse) can result in major abortions storms. Determine if single nucleotide polymorphisms (SNPs) in IBRV can be used as a method to resolve whether outbreaks were likely to be iatrogenic (vaccine induced).

Materials and Methods: To determine the SNP pattern of a virus, the entire virus must be sequenced and then the vaccine viruses matched up with a "known" laboratory virus. Using methods developed by Fulton et al, 2013 (Fulton, R. W., d'Offay, J. M., & Eberle, R. Bovine herpesvirus-1: Comparison and differentiation of vaccine and field strains based on genomic sequence variation. 2013. Vaccine, 31(11), 1471-1479). The sequences were then compared and SNPs were identified. Using this information 3 different segments were compared and individual SNP were identified. The unknown virus isolate is then compared to the known SNP for the vaccines. This analysis was done on either virus isolates or infected tissues (either placenta or fetus).

Results: We have had 15 abortion episodes (10 from Wyoming, 2 North Dakota, 2 from Minnesota and 1 from Indiana) where we have virus isolates between 2010- 2015 where an apparent association existed between use of modified live BoHV-1 and abortion in the subsequent 1-8 months. In individual episodes the products were either used "on or off label" according to the producer. All 15 isolates had SNP patterns

consistent with those of commonly used modified live BoHV-1 strains. Use of SNP patterns is helpful in resolving whether abortion was likely due to "vaccinal virus" particularly when disagreement existed between a producer and representatives of the vaccine manufacturer

Conclusions: Use of vaccines containing MLV BHV-1 in has inherent risks to reproductive fitness. Fifteen different cases were tested using SMPs. These cases reinforce the need to make sure the cow herd is adequately vaccinated if calves are vaccinated with MLV vaccine while still with the cows. However when "properly vaccinated" heifers have higher susceptibility to MLV induced abortion. In summary, MLV BHV-1 vaccines must be used judiciously following label directions. In spite of the following the label, reproductive issues can still occur.

IBR

P02-002-233

Severe Bhv-1 Outbreak In A Naive Dairy Herd In Northwest Spain

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Objectives: The commercial dairy herd is located in Galicia (North West of Spain), a densely populated region with farms that average a capacity of 40 cows. This is a large herd with 400 dairy cows (250 milking) and it is engaged in a voluntary regional health program for the control and eradication of IBR, BVD, Neospora and Johne's disease. This farm has regularly vaccinated for BVD and it was considered to be free of IBR according to yearly serology control for IBR and bulk tank milk analysis followed in the region.

Materials and Methods: In June 2014 this farm was presented with a respiratory outbreak that was mainly affecting the adult animals. Pyrexia (40°C-40,5°C), anorexia, dyspnea, cough and nasal discharge, firstly serous and then muco-purulent, also combined with conjunctivitis and ocular discharge. One animal died and at post-mortem it was evidenced pneumonic consolidation and emphysema. The average milk production of the herd was decreased by 2 liters per animal. Young suckling calves were also affected, and the group of calves showed the same respiratory clinical signs, and of those one 4 month old calf died few days later.

Results: All the affected animals were first treated with nonsteroidal anti-inflammatories, marbofloxacin and ceftiofur, although little success was found. One week after the symptoms appeared it was decided to do an intranasal vaccination against BRSV and P-I3 in milking animals, and 10 days later that same vaccine protocol was extended to the whole herd. The farmer, due to the poor response to the vaccination decided then to contact the veterinarian of the regional health program.

The veterinarian did the anamnesis, farm inspection and clinical examination and he finally suspected of an IBR outbreak as the main cause of the problem. He then took some nasal swabs for virus detection (IBR, BRSV, BVD) by PCR (Boviresp FTA®, Hipra) (1). This analysis came back as positive for IBR and some days later it was evidenced IBR seroconversion in blood samples from 12 to 24 months old heifers and also in milk samples from the bulk tank.

Conclusions: In front of the evidence of the lab results obtained the veterinarian decided the introduction of an MLV IBR marker vaccine gE-/tk- deleted (Hiprabovis® IBR Marker Live, Hipra) to the whole herd. The symptoms then began to decrease in just 48h after vaccination, no more

animals became sick after the introduction of the vaccine and the milk production went back to normal levels.

Comments: Currently this herd is on a six monthly program with HIPRABOVIS® IBR Marker Live protocol for the prevention of IBR, with a double primo-vaccination in the young heifer calves at 3 months of age and booster vaccinations in the adults every 6 months. The farm IBR status is regularly monitored by bulk milk tank sampling every 6 months and every 12 months by the sampling and IBR gE serology of 12- 24 months old heifers. No more clinical cases of IBR have been detected and the epidemiologic situation of IBR is now under control.

IBR

P02-002-234

Reduction in daily milk yield associated with sub-clinical Bovine Herpes Virus 1 infection

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Objectives: Bovine Herpesvirus 1 (BoHV-1) incursion into a naïve population of adult dairy cows typically leads to a variety of clinical syndromes described as Infectious Bovine Rhinotracheitis (IBR). BoHV-1 may also persist and lead to sub-clinical disease with insidious production losses and potentially serious economic consequences. The longer term effects on milk production of sub-clinical BoHV-1 remain unknown and it is important in understanding the relative importance of IBR control. This study aimed to investigate the effect of sub-clinical BoHV-1 infection on milk production over a 2 year period on a UK dairy herd.

Materials and Methods: Infection status of cows (infected or not infected) in a closed autumn-calving dairy herd was assigned from serology on a single occasion in May 2010. We infer nothing about the temporal dynamics of virus circulation in the herd, other than the observed change in herd classification based on bulk milk serology. The herd comprised of 129 pedigree Holstein cows with approximate annual milk yield of 9,000 kg per cow. A multi-level linear model was used to evaluate the impact of infection status on milk production, using milk records that were routinely collected over two years from January 2009 to December 2010. Based on bulk milk serology and intermittent blood samples from young stock up to February 2010, the herd was assumed previously uninfected with BoHV-1.

Results: Seventy two per cent of cows were seropositive to BoHV-1 on individual blood samples taken in May 2010. Risk of sero-conversion varied with parity, with a higher proportion of parity 1 and >4 testing positive. Importantly, cows that were seropositive to BoHV-1 in May 2010 produced 2.6 kg per day less milk throughout lactation compared to those that were seronegative. This was further highlighted through the production of a mean predicted lactation curve for parity one cows in December with mean milk fat, protein and somatic cell counts based on BoHV-1 antibody status. It clearly demonstrated that peak lactation was reached at the same time point, irrelevant of antibody status, but animals that were seropositive to BoHV-1 peaked at a considerably lower milk yield. The decline in yield over the course of lactation was predicted to occur at the same rate, irrelevant of antibody status, indicating that those that were seropositive to BoHV-1 maintained a considerably lower yield throughout lactation. Compared to seronegative cows, those cows with

antibodies to BoHV-1 on average failed to produce almost 1,000 kg of milk per year.

Confounding factors influencing test day milk yield were calendar month, parity, stage of lactation, test day fat and protein percentage and somatic cell count. Residuals from the data were normally distributed indicating a good fit to the data.

Conclusions: Sub-clinical BoHV-1 infection has been associated with a large decrease in potential daily milk yield. This potential fall in production is larger and predicted to last longer than previous estimates. This variation could relate to differences between studies, disease dynamics, BoHV-1 strain or the analysis. The large potential losses in milk production highlights the importance of herd health management to prioritise management interventions such as; biosecurity and vaccination. It also highlights the vitality of effective monitoring to mitigate the effects of sub-clinical disease.

Reference: Veterinary Record

IBR

P02-002-235

Preparation and first 4 years of the mandatory IBR-program in Northern-Belgium

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Objectives: IBR (infectious bovine rhinotracheitis) is caused by BoHV-1. Starting from January 5th 2012, participation to the national IBR-eradication programme became mandatory in Belgium. In the year 2010-2011, all cattle owners in Belgium are preparing for IBR-control either through vaccination or blood sampling. This abstract describes the “starting-up” phase of the national IBR-campaign in Northern-Belgium and the experiences of the first 4 years of the program from a perspective of animal health care Flanders.

Materials and Methods: Cattle owners in Belgium can obtain an IBR-‘status’ either through marker-vaccination or through blood sampling in animals for IBR antibodies. To start IBR-control, farmers were advised to determine their IBR status through an initial herd sample consisting of approximately 26 ‘representative’ animals, analysed for gE or gB antibodies (Idexx). After interpretation of this herd-sample and/or combined with herd anamnesis, it is decided which status is to be obtained: ‘12’-status (mandatory vaccination), ‘13’-status (free of wild-virus) or ‘14’-status (free of both virus- and vaccine- antibodies). Free-status (13/14) is obtained after two consecutive negative gE/gB screenings (all animals above 12 months). Status has to be maintained through yearly blood sampling, bi-annual vaccination and is accompanied by biosecurity restrictions. Herds without a status are categorised as ‘11’ and will be blocked in 2012 (limited to removal of animals directly for slaughter).

Results: In this abstract, the situation until October 2015 is given. At the beginning of the program in 2012 2.900 cattle herds obtained a ‘13’ or ‘14’ status (free of BHV-1). About 8.300 herds performed an initial herd sample to determine their infection status. 72% of all vaccinating herds (‘12’-status) never sampled their animals to determine their IBR-infection status. In October 2015, the number of herds obtaining a free status was 5.068 or 33% of cattle herds in Northern-Belgium. Results indicate that though systematic control the between-herd prevalence and within herd prevalence of BHV-1 is diminishing.

Conclusions: The evolution of IBR-control, restraints and the experienced difficulties of the “starting-up phase and first 4 years will be discussed. Focus will lie on farmers and herd veterinarians choices (vaccination or obtaining a free status) and motivators in a mandatory IBR-control, coupled with the communication campaign conducted at Animal Health Care Flanders to guide Northern-Belgian cattle owners to a form of IBR-control.

IBR

P02-002-236

Interim Report of a Long-term BoHV-1 Eradication Program in Polish Dairy Cattle Herds - Success and Risk

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Objectives: Bovine herpesvirus 1 (BoHV-1) causes infectious bovine rhinotracheitis (IBR), abortions and other syndromes in cattle. In many parts of the world, vaccination is used as an important tool in BoHV-1 control and eradication programs. With parts of Europe now IBR free, the ability to differentiate infected from vaccinated animals (DIVA) has become critical as a trading tool. For this purpose, modified-live and inactivated gE deletion marker (gE negative) vaccines were developed. The objective of this study is to evaluate the efficacy of different DIVA vaccination protocols to aid in the eradication of BoHV-1 from Polish dairy cattle herds.

Materials and Methods: A total of approximately 5000 cattle from 5 dairy farms were included in the study in January 2014. All farms had already started eradication programs between 2005 and 2013. During the study, two commercial vaccines, a modified live vaccine [Risposal[®] IBR Marker Vivum (VV); Zoetis] administered intranasally (IN) or intramuscularly (IM) and an inactivated vaccine [Risposal[®] IBR Marker Inactivated (VI); Zoetis] administered subcutaneously, were used as follows:

Farm A (1300 animals): VI at 3-4 months of age and again after 3-4 weeks, then VI at 6 monthly intervals.
 Farm B (800 animals): VI at 6 months of age and again after 3-4 weeks, then VI at 6 monthly intervals for 2 years followed by yearly intervals.
 Farm C (1200 animals): VI at 6 months of age and again after 3-4 weeks, then VI at 6 monthly intervals for 3 years followed by yearly intervals.
 Farm D (800 animals): VV at 2 months of age IN and after 3-5 weeks IM, then VI at 6 monthly intervals for 2 years followed by yearly intervals.
 Farm E (690 animals): VV at 6 months of age IM, then VI after 6 months, followed by VI every 12 months.

To determine BoHV-1 seroprevalence, serum samples were collected from study animals at 7-9 months of age, 12-13 months of age and at 1st, 2nd 3rd etc. lactation. In cows serum samples were collected from randomly selected 20% of animals. Samples were analysed by ELISA (HerdCheck BHV1 gE; IDEXX, Scandinavia AB, Sweden) for antibodies against the gE protein of BoHV-1.

Results: The ELISA results confirm, that the percentage of animals with antibodies to BoHV-1 gE has been decreasing in each of the vaccinated herds over time. In Farm A (gE-positive animals: 79% in 2013, 43% in January 2014, 35% in December 2014, 14% in October 2015), seroprevalence decreased continuously due to vaccination and culling

of gE-positive animals. On Farm B (gE-positive animals: 100% in 2005, 0%, In January 2014, 0% in December 2014, 0% in October 2015) and on Farm C (gE-positive animals: 100% in 2006, 5% in January 2014, 0% in December 2014, 0% in October 2015), the BoHV-1 free status is maintained through 6-monthly vaccinations with the inactivated vaccine. On Farm D (gE-positive animals: 90% in 2008, 76% in January 2014, 48% in December 2014, 48% in October 2015 with 0% of gE-positive animals in the age group 7-13 months), continuous improvements in eradication are observed as replacement animals grow up BoHV-1 free and older animals are removed from the herd. On Farm E (gE-positive animals: 100% in 2013, 78% in January 2014, 53% in December 2014, 27% in October 2015), heifers from different sources were brought into the herd in late autumn 2014, without quarantine procedures or serological testing. Subsequent serological testing showed that the decrease in the percentage of gE-positive animals in the herd had slowed down, and counter measures such as culling were implemented to prevent a BoHV-1 outbreak.

Conclusions: The interim results of this study lead to the following conclusions:

1. DIVA vaccination programs with live and inactivated BoHV-1 (Risposal IBR Marker) vaccines are an effective tool in the eradication of IBR in dairy cattle herds.
2. Continuous serological testing especially of young animals is recommended.
3. Good management practices such as identification of BoHV-1 gE-positive animals and biosecurity measurements are essential for the success of BoHV-1 eradication programs.

IBR

P02-002-237

Control and Monitoring of Infectious Bovine Rhinotracheitis in a Closed Dairy Herd Following Vaccination with Live/Inactivated IBR Marker Vaccine

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Objectives: An opportunity arose to study the epidemiology of IBR in a closed (apart from occasional stock bull purchase), 230 cow Holstein dairy herd. Following an IBR breakdown in 2012 the breeding herd was vaccinated with a live/inactivated IBR marker vaccine programme with the aim of controlling clinical disease and reducing risk of subsequent virus shedding from latently infected animals. The herd was closely monitored by individual milk/serum IBR antibody testing over a 4 year period to follow IBR infection status with a view to achieving eradication of IBR from the herd.

Materials and Methods: The herd had excellent boundary biosecurity and homebred heifers were reared on the same farm as cows but housed in separate accommodation until pregnant. Vaccination for IBR had been carried out 2002-2007 (marker vaccine used 2006/7) but ceased in October 2007. IBR status was then monitored by annual, first lactation heifer cohort, milk IBR antibody testing.

In May 2012, a milking heifer showed clinical signs of IBR and died, with IBR confirmed by PCR testing and histopathology.

Herd status was investigated by testing all cows for milk or blood IBR gE antibody (IDEXX Elisa) to define herd seroprevalance. Bulling and in-calf

heifers plus the stock bull (IBR free at purchase) were blood tested for IBR gB antibody (Biobest indirect ELISA).

The breeding herd (all cows, pregnant and bulling heifers) were vaccinated i/m with a live IBR marker vaccine (Risposal® IBR Marker Live ,Zoetis) followed 4 weeks later by an inactivated IBR marker vaccine (Risposal® IBR Marker Inactivated ,Zoetis) s/c and boosted annually with inactivated IBR vaccine. In subsequent years the bulling heifers received a primary vaccination course of live IBR vaccine i/m followed 4 weeks later by inactivated IBR vaccine before breeding and then boosted annually with inactivated IBR vaccine. Monitoring continued with annual individual IBR gB antibody blood testing of bulling heifers prior to vaccination, IBR gE antibody blood testing of in-calf heifers and individual milk IBR gE testing of cows from 2012-2015. At the 2014 test both milk and blood samples were taken from cows on the same day.

Results: First lactation heifer cohort milk IBR gB antibody testing between 2008 and 2011 proved negative suggesting absence of active infection in the milking herd during this period.

The individual IBR antibody percentage sero-positivity for herd groups for years 2012-2015 respectively were :

Bulling heifers : 1%(n=90), 0%(n=105), 0% (n=96) and 0% (n=119).

In-calf heifers : 68%(n=69), 1%(n=90), 0%(n=105), not tested in 2015 but all negative as bulling heifers.

Milking cows : 99%(n=230), 91%(n=228), 63%(n=235) and 45%(n=226).

The stock bull tested IBR antibody positive in 2012 having previously been confirmed IBR free at entry to herd.

The sampling results showed that in 2012 IBR appeared to have spread widely in the milking herd and the in-calf heifers that had been mixed with dry cows. In the six months prior to this the herd had experienced no "classical" signs of IBR but poorer than average herd fertility had been noted. Following vaccination of the breeding herd the subsequent replacement heifer cohorts have remained IBR free to date. The cow herd seroprevalance fell from 99% to 45% in a 3 year period with normal culling policy.

The comparison of individual milk and serum IBR gE (IDEXX Elisa) cow test results done in 2014 showed reasonable correlation but suggested false positives are likely with a single milk gE test sample. The correlation for 213 milk/blood sampled cows were as follows :

Milk gEpos/serum gE pos	128
Milk gE neg/serum gE neg	74
Milk gE pos/serum gE neg	10
Milk gE neg/serum gE pos	1

Conclusions: Within 5 years of ceasing an IBR vaccination programme the disease appeared to recrudescence and spread widely in this herd.

Vaccination with a live/inactivated IBR marker vaccine programme has to date controlled clinical disease and possibly reduced the risk of latent carrier cows shedding virus and infecting naïve heifers.

Within 3 years the adult cow herd seroprevalance has fallen from 99% to 45% suggesting with current replacement/cull rates eradication could be achieved within 5-6 years of initial diagnosis by removing residual latent carriers.

Immunology/Vaccinology

P02-002-132

Application of a new method for MHC class I immunotyping applying RNA-Seq with regard to the aetiopathogenesis of bovine neonatal pancytopenia (BNP)

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Objectives: Bovine neonatal pancytopenia (BNP) is an alloimmune-mediated disease, which is induced by ingestion of colostral alloantibodies from some dams, which have been vaccinated with a specific vaccine. It has been proposed that after vaccination contaminating MHC class I from the producer cell line (Madin-Darby bovine kidney cell line, MDBK) of this vaccine elicit alloantibody production against MHC class I in some dams (BNP-dams). No specific, causative MHC class I alleles for BNP have been identified. Additionally, the Immuno Polymorphism Database (IPD-MHC BoLA) contains only a limited number of published MHC class I sequences for *Bos taurus*.

Materials and Methods: In consideration of the limited MHC class I allele catalogue and complex MHC class I haplotypes in cattle, we developed a new strategy for immunotyping of classical and non-classical MHC class I alleles at nucleotide level. High-throughput mRNA sequencing from whole blood samples of dams with divergent BNP phenotypes and the MDBK cell line was carried out as a paired-end, 2x61bp approach. Alignment of reads obtained from sequencing was performed with different Bowtie options. A catalogue of previously published MHC class I alleles served initially as reference sequence. Applying less stringent alignment options subsequently enabled deduction of novel alleles from previously published alleles. The initial allele catalogue was extended with newly discovered alleles for further and final alignment. The method additionally enabled quantification of MHC class I expression on allele level. Expressed MHC I alleles of BNP-cows, non-BNP cows and the MDBK cell line were compared with respect to a potential causative BNP allele.

Results: We obtained a comprehensive list of expressed classical and non-classical MHC class I alleles, including previously published alleles and several, previously unpublished alleles. Expression of identified MHC class I alleles could be confirmed by Sanger sequencing. The detection of novel MHC class I alleles provides further sequence information for a currently incomplete MHC class I sequence set in cattle. MHC class I alleles expressed by BNP-dams, control dams and the producer cell line of the vaccine were subsequently compared at nucleotide level as well as on amino acid level. This enabled us to gain further insights into the aetiopathogenesis of BNP. No specific MHC class I allele or amino acid position could be detected as BNP causative allele or amino acid position. Regarding the quantification of MHC class I alleles, expression levels of single assigned alleles varied within individuals, while the relative proportion of reads assigned to alleles was strikingly constant.

Conclusions: Our results show that our new MHC class I immunotyping strategy is applicable to detect unknown and already published MHC class I sequences in cattle. Regarding BNP, application of the novel approach has provided evidence that specific classical and non-classical MHC class I can be excluded as single causal agents for BNP-associated alloantibodies.

Immunology/Vaccinology

P02-002-133

Effectiveness of a new recombinant immunocastration vaccine in bovine

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Objectives: Animal castration for meat production is a widely used method since it allows the inhibition of sexual and aggressive behavior of animals. Recently, a new technology has been developed called immunocastration, which through the generation of neutralizing antibodies against the hormone GnRH, is able to block temporarily the hormone activity, preventing the production and release sex steroids. The aim of this work was evaluate a new recombinant immunocastration vaccine for use in cattle in a field clinical trial to evaluate safety and efficacy, by determining its effect on a number of hormonal, behavioral and production parameters.

Materials and Methods: The vaccine composition will be based on the use of a recombinant antigen of the hormone GnRH-I (EP 2431052 B1) and an adjuvant based on low molecular weight Chitosan. A group of 90 animals were vaccinated with GnRX G/Q to 11 months old followed by a new dose 30 days after the first application. The control group was castrated surgically at same time. The clinical trial was evaluated using the standards of Good Clinical Practices during 6 months. Serum samples were taken at day -30, 30, 80, 122 and 165 to evaluate testosterone levels and specific rise of immunoglobulins against GnRH. Productive parameters as final weight, dressing, quality of meat and sexual behavior were evaluated. Two-way ANOVA was used as statistics test.

Results: The rise of levels of testosterone was blocked after the second doses and remain low until the 165 day, around 2 ng/ml. The sexual behavior had an increase at day 80, but this fell to day 165 with an increasing of social and agonistic behavior. The final weight was 5% higher in the immunocastration group with 1% less dressing and butter weight of carcass than control group. The quality of meat was similar in fat infiltration, pH and tenderness in both groups. Testicular histology showed a reduction of spermatogenesis in tubules and absence of spermatozoa in epididymis.

Conclusions: This new recombinant vaccine was effective in reducing testosterone levels after two vaccinations, generating a blocking spermatogenesis and sexual activity for 6 months. The quality of meat from animals immunocastrated was very similar to that of surgically castrated animals. The immunocastration achieved a slight but significant increase in productive parameters such as weight and final weight of carcasses.

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Immunology/Vaccinology

P02-002-135

Vaccination against clostridial disease in maternally derived antibody positive lambs; a randomised, non-inferiority field trial in twin lambs.

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Objectives: In New Zealand (NZ) prevention of clostridial disease by vaccination of ewes 2-6 weeks pre-lamb normally protects lambs until weaning; however, in some flocks, *Clostridium perfringens* type D (i.e. Pulpy Kidney) deaths still occur. Additional vaccination at tailing (~4 weeks old) is used to reduce this risk. As most lambs born to vaccinated dams will have maternally derived antibodies (MDA), it is crucial that a vaccine is efficacious in the face of MDA. This study examined the immune response in MDA positive lambs, vaccinated at tailing.

Materials and Methods: A randomised parallel group, non-inferiority field trial, conducted on a sheep and beef farm in Masterton, NZ. In July 2012, 126 maiden, twin-bearing, Romney, 2-tooth ewes were injected with a single dose of the test vaccine (n=63, Multine™ 5, MSD Animal Health NZ) or the reference vaccine (n=63, Ultravac® 5 in 1, Zoetis NZ). Following randomisation, each lamb within 50 sets of twins was vaccinated with the test or reference vaccine at tailing (V1- aged 26 to 53, median 36 days) and again in 49 days at weaning (V2). Epsilon and tetanus antitoxin concentrations were determined by ELISA at V1 (MDA) and at V2+14 days. Data were reported as geometric mean concentration (GMC) and the geometric mean ratio (GMR) of the test/reference and their [95% confidence interval (CI)]. Failure to respond was defined as a decrease in antitoxin concentration at V2 + 14 relative to V1. The European Pharmacopoeia (EP) vaccine release concentration of 5 and 2.5u/ml for epsilon and tetanus respectively was also used to assess individual response. Non-inferiority was assessed by calculation of the lower limit of the one-sided CI of the GMR at V2 + 14. Non-inferiority was declared if this lower limit was ≥ 0.63 .

Results: Epsilon and tetanus MDA, 0.3-10.5 & 0.1-11.5u/ml resp., were detected in all lambs at V1. Lambs from ewes that got the test vaccine had higher epsilon MDA than the reference (GMC 3.0 u/ml, n = 59 and 1.4 u/ml, n = 60, respectively; GMR 2.1; P < 0.0005). The MDA tetanus GMC was 1.7 and 1.3 u/ml in test and reference groups resp.; GMR was 1.3 (P = 0.056). Epsilon antitox. increased in 13/19 of control lambs indicating some natural exposure prior to V2 +14. Nevertheless, 11/50 lambs with the reference failed to respond; whereas all reference lambs responded. MDA conc. at V1 was neg. associated (P = 0.004) with no response in the reference group; the response % being 100 % with MDA at the 25th perc., compared with 74 % and 30 % at the 75th and 90th perc. resp.. Most (48/50) lambs vaccinated with the test had epsilon antitox. conc. > EP standard, compared with 30/50 of reference lambs (adjusted margin 36%, P < 0.0005). The test product was non-inferior to the reference (GMR lower limit 1.9). Moreover, the test vaccine induced a 2.5x greater mean response (GMR 2.5, P_{Bonf} < 0.0005). Tetanus antitoxin increased in 49/50 and 41/50 of the reference and test lambs resp., no evidence of natural exposure. No response in the reference group was (p=0.005) associated with the MDA conc. at V1. Most (49/50) test lambs had tetanus antitox. > EP standard; compared to 42/50 of the reference lambs (adj. margin 14%, P = 0.010). The test product was non-inferior to the reference (GMR lower limit 1.3). Moreover, the test vaccine induced a 1.8x higher mean response (GMR 1.8, P_{Bonf} < 0.0005).

Conclusions: All lambs had epsilon (Pulpy Kidney) and tetanus MDA at tailing indicating successful colostral transfer. Lambs born to ewes vaccinated with test vaccine had higher MDA, than lambs born to ewes vaccinated with reference vaccine. The test vaccine overcame maternal antibodies and induced a protective immune response, superior to the registered reference product. The test vaccine can be prescribed for use in lambs at tailing when these lambs are born to ewes which have had a clostridial vaccination prior to lambing.

Immunology/Vaccinology

P02-002-136

Antibody to leukocidin mitigates the mastitis induced by exosecretion derived from predominant lineage of *S. aureus* isolated from bovine mastitic milk

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Objectives: *Staphylococcus aureus* (*S. aureus*) is a common etiological agent of bovine mastitis. The specific factors involved in mastitis and its effective treatment are not well understood. We investigated the relationship between *S. aureus* exosecretions and mastitis. We conducted an epidemiologic analysis by comparing genetic variation among *S. aureus* isolates from bovine milk, estimated the inflammatory effect of exosecretions derived from epidemiologically predominant strains both in vitro and in vivo. We further conducted a passive immunization study evaluating the effectiveness of humoral immunity against the inflammatory effects of exosecretions.

Materials and Methods: A total of 97 *S. aureus* isolates from the milk of infected bovine mammary glands collected from 2011 to 2014 from 12 farms in Japan were included in this study. The genes encoding leukocidins (LukM/F, LukE/D, hlg, LukPVL), Sag, enterotoxins (A-I), and TSST were detected using PCR to monitor the variety of exosecretions. *S. aureus* capsular typing and multilocus sequence typing (MLST) were also performed to evaluate the bacteriological lineage. In order to evaluate the leukotoxic activity of exosecretions with different leukocidin profiles in vitro, supernatants of *S. aureus* cultured in brain heart infusion broth were inoculated to granulocytes derived from bovine peripheral blood in microtiter plates. Leukotoxic activity of each specimen was determined as the highest dilution of supernatants, indicating a flattened effect against granulocytes. Supernatants were purified via two step ammonium sulfate precipitation followed by filtration and inoculated into bovine udders. The degree of inflammation was evaluated via somatic cell counts (SCC). A passive immunization study was conducted by pre-incubating LukM/F positive *S. aureus* exosecretions with immune serum, which neutralized the leukotoxic activity before inoculation into the bovine udder. The expression of cytokines (IL-1, IL-6, IL-8, and TNF- α) by milk cells was additionally characterized using real-time RT-PCR to estimate the degree of inflammation.

Results: Almost all the *S. aureus* strains derived from bovine mastitis possessed LukE/D and hlg genes, and 54% of the strains possessed the LukM/F gene. In contrast, enterotoxin, PVL, and Sag genes were rarely found. The dominant *S. aureus* lineage, Capsular type 5 - MLST CC97, was strongly correlated with the presence of LukM/F genes. Exosecretion of LukM/F positive strains showed significantly higher cytotoxic activity against bovine granulocytes in the range of 800-6400 compared with exosecretion of LukM/F negative strains (<10-40). Both semi-purified exosecretions of LukM/F positive and negative strains induced mastitis 8 to 24 hours after inoculation into bovine udder, but the clinical signs were significantly greater with the former specimen (SCC= 467 x10⁴ and 106 x10⁴ /ml of the peak). This inflammatory effect was mitigated by pretreatment with immune serum in a dose dependent manner. Cytokine gene expression was coincidentally suppressed, with the exception of IL-8, by pretreatment with immune serum.

Conclusions: The predominant lineage of *S. aureus* isolated from bovine mastitis efficiently expressed LukM/F, and its exosecretions caused inflammation both in vitro and in vivo. We further suggested the possible beneficial effect of humoral immunity against exosecretions, including leukocidins, to support the host defense mechanisms during bovine mastitis. Further studies are needed to assess the expression of leukocidins in the bovine udder and to characterize their pathological role in mastitis.

Immunology/Vaccinology

P02-002-137

Safety of Mucosiffa administered to pregnant dairy cow

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Objectives: Transplacental infection of the foetus during the first trimester of gestation by a non-cytopathogenic biotype of BVDV has been shown to induce reproductive disorders (embryonic resorption, abortion) as well as provide the potential for the generation of Permanently Infected calves (PI).

This study was carried out to demonstrate the safety of a high dose of MUCOSIFFA (live attenuated vaccine against BVDV) when administered to pregnant dairy cows and heifers representing all stages of gestation. The study design used is in line with that recommended by the European Pharmacopoeia.

Materials and Methods: The study was a blinded, randomized and negative controlled trial. 82 pregnant females (cows and heifers) representing all trimesters of gestation were included, with vaccinates and controls equally represented in each trimester. There were 30 first trimester cases, ranging from 30-120 days in calf, 28 second trimester cases ranging from 121-200 days and 24 third trimester cases which were all >200 days in calf. The vaccinates each received a single administration of twice the maximum release dose of the vaccine in many countries (i.e. receiving 6.3 log₁₀ CCID₅₀ per 2 mL dose).

The potential safety of the vaccine was evaluated by monitoring the number of successful calvings, rectal temperatures, local and systemic reactions as well as milk production. The health and BVDV status of the offspring were also monitored for 2 weeks post-calving.

Results: Both the vaccinate and control groups recorded 95% of successful gestations (78/82 cows, producing 83 calves). Overall, 82 out of the 83 calves (99%) were born in good health, with one calf from a control animal dying within two days of calving. None of the calves were detected as PI.

There were no local or systemic reactions observed following the vaccinations. None of the animals in the study recorded a temperature above 39.1°C during the monitoring periods.

Milk production (monitored daily one week before and one week after vaccination) was recorded daily by a milking robot (De Laval). No statistical difference between vaccinated and control animals was recorded (ANCOVA, p=0.151).

Conclusions: There was no observable negative effect of the vaccine on the reproductive performance of the vaccinated cows and heifers and similarly no observable negative effect on the health status of the calves born from vaccinated animals. The safety of a high dose (6.3 log₁₀ CCID₅₀) MUCOSIFFA administered once during any stage of gestation was confirmed for the vaccinated cows and for their foetuses.

Immunology/Vaccinology

P02-002-138

Identification of candidate protein biomarkers for BPI3V diagnosis using an in vitro infection model

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Objectives: The viral pathogen Bovine Parainfluenza Type 3 Virus (BPI3V) of the Bovine Respiratory Disease complex often manifests in an asymptomatic infection, but causes respiratory tract damage, suppressing innate and adaptive immune responses, facilitating proliferation of endogenous opportunistic pathogenic bacteria. Routine BPI3V diagnosis is performed using ELISA, however, positive antibody response does not indicate damaged respiratory tissue or active infection. This study aimed to apply an in vitro biomarker screening approach to identify markers of BPI3V infection for diagnosis of active infection in vivo.

Materials and Methods: Foetal Calf Lung (FCL) fibroblasts were infected with BPI3V at a multiplicity of infection of 10 for 1h and washed with GMEM. 2D Gel Electrophoresis was performed using lysates prepared from BPI3V-infected and mock-infected FCL cells at 0h, 24h and 48h post-infection (p.i.) to identify potential diagnostic markers of BPI3V infection in vivo. The presence of in vitro selected markers was investigated in the blood of BPI3V infected animals using targeted multiple reaction monitoring (MRM) ultra-performance-liquid-chromatography tandem-mass-spectrometry (UPLC-MS/MS). All animal studies were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and with the approval of the AFBI Ethical Review Committee. 12 male Holstein Friesian calves aged between 20 and 25 weeks were divided into two cohorts (n=6) designated as either non-vaccinated or vaccinated study groups. Vaccinated group animals were treated with Pfizer Rispoval® PI3+RSV intranasal vaccine as per manufacturer's instructions, and non-vaccinated group animals with empty poly(lactico-glycolic) acid (PLGA) nanoparticles. Plasma taken from vaccinated and non-vaccinated BPI3V challenged animals at day 0 and days 1, 2, 5, 6, 14 and 20 post-challenge (post-BPI3V) was subjected to tryptic digestion and MRM UPLC-MS/MS analysis for the quantification of unique peptides corresponding to in vitro selected protein biomarkers.

Results: The levels of 35 proteins were found to be significantly altered in FCL cells as a result of BPI3V infection. BPI3V induced alterations to the intracellular proteome of respiratory FCL fibroblasts differed to that typically observed in A549 respiratory epithelial cells (apoptotic), resulting in elevated host-proteins associated with mRNA translation, protein translation, post-translational modification and cellular protein trafficking. Phosphoprotein P, T-complex protein 1 subunit-theta and 14-3-3 protein, found to be significantly accumulated within BPI3V infected FCL cells in vitro, were selected as potential diagnostic markers of BPI3V in vivo infection. Using targeted MRM UPLC-MS/MS, proteotypic peptides for Phosphoprotein P and T-complex protein subunit theta were quantified in plasma at days 0, 1, 2, 5, 6, 14 and 20 post-BPIV-3 challenge in vaccinated or non-vaccinated study groups. However, no peptides corresponding to 14-3-3 protein theta were detected in plasma. Unique peptides for phosphoprotein P and T-complex protein 1 subunit theta were found to be significantly (p<0.05) up-regulated at day 5 post-BPI3V in non-vaccinated animals compared to vaccinated animals at the same stage. Plasma levels of Phosphoprotein P were found to increase significantly (p<0.05) from day 1 to day 5 post-BPI3V in non-vaccinated animals. A significant increase in the levels of T-complex protein 1 subunit theta was observed from day 0 to 20 post-BPI3V in plasma of vaccinated animals.

Conclusions: This study demonstrated that proteomic analysis of BPI3V infection in vitro is capable of selecting markers for in vivo BPI3V diagnosis. Viral (Phosphoprotein P) and host-proteins (T-complex protein 1 subunit theta) accumulated intracellularly at the latter stages of infection are released into the circulating bio-fluids, with elevated levels occurring in non-vaccinated BPI3V challenged animals at periods associated with peak virus titre. Compared to serological ELISA,

assessment of such markers released from infected cells or diseased tissue would provide increased information on the health status of serologically positive animals.

Immunology/Vaccinology

P02-002-139

LPS and the NLRP3 inflammasome activator nigericin drive enhanced IL-1 β expression in bovine uterine endometrial cells

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Objectives: Postpartum bacterial infection of the uterus occurs in all cows, resulting in an inflammatory response associated with healthy involution. However, 30% of cows will develop endometritis, a period of pathological uterine inflammation, with consequences for fertility. Little is known about this transition to pathological inflammation. Here we propose a critical role for endometrial cells in mediating this switch and hypothesize that regulation of inflammasome activation is key. The aim of this study is to investigate the role of inflammasome activated IL-1 β (along with other members of the IL-1 family, namely IL-1 α /IL-18) in uterine inflammation

Materials and Methods: Bovine endometrial tissue was isolated from non-pregnant bovine uteri at stage I of the oestrus cycle, immediately post-mortem. Epithelial and stromal cells were isolated from the uterine horn ipsilateral to the corpus luteum. Alternatively, 8mm biopsy punches were taken for explant culture. Pure populations of cells were confirmed based on morphology and on positive staining for cytokeratin (epithelial) and vimentin (stromal). Absence of CD45⁺ immune cell contamination was confirmed using PCR. Structural integrity and viability of the explant cultures was investigated using histology and by measuring LDH activity in the culture supernatants. To investigate IL-1 production, explant and cell cultures were stimulated with LPS for 3, 6, 12 and 24 hours, followed by nigericin (an inflammasome stimulator) for 1 hour. Levels of IL-1 α , IL-1 β and IL-18 mRNA were measured using qRT-PCR and protein levels of IL-1 β were measured using an ELISA.

Results: Cell isolations (n=6) yielded an average of 1.2×10^6 cells/uterine horn. Cell populations were ~90% pure based on morphology and positive staining for vimentin (stromal) and cytokeratin (epithelial). Histological sections of explant cultures showed normal tissue architecture and no significant differences were observed in LDH activity between control and LPS stimulations (n=7). Stimulation of epithelial and stromal cells (n=6) indicate that bovine endometrial cells produce IL-1 α and IL-1 β in a time dependent manner, with mRNA levels peaking after 6 hours. Levels of IL-1 α mRNA were higher in stromal cells (mean fold change=99.5) than in epithelial cells (mean fold change=1.2) at 6 hours, with IL-1 α mRNA levels in the explant cultures (n=7; mean fold change=3.5) remaining close to the levels seen in the epithelial cells at 6 hours. Similarly, levels of IL-1 β mRNA were significantly higher in stromal cells (mean fold change=86.9, p=0.0062) than in epithelial cells (mean fold change=1.7) at 6 hours, with IL-1 β mRNA levels in the explant cultures (n=7; mean fold change=1.6) closely mirroring those of the epithelial cell cultures at 6 hours. This was verified at a protein level using an ELISA, with nigericin required for protein production. In

contrast, the expression of IL-18 mRNA was not induced in response to LPS and nigericin but was found to be constitutively expressed across all cultures and time-points.

Conclusions: This is the first study to show a novel role for the inflammasome mediated IL-1 β cytokine in the bovine endometrium. The differing levels of IL-1 α and IL-1 β produced by epithelial and stromal cells suggests a principal role for the stromal cells in the orchestration of the inflammatory immune response. On-going work will aim to examine the anti-inflammatory potential of these cells and will also aim to utilise inflammasome inhibitors to characterise the pathways mediating IL1 β production. Shedding light on the mechanisms regulating inflammation will ultimately lead to identifying novel routes to therapeutic intervention for endometritis

Immunology/Vaccinology

P02-002-140

Diathelic immunization of heifers against E.coli improves responsiveness to experimental mastitis in early lactation

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Objectives: Escherichia coli is a major causal agent of mastitis that impacts in multiple ways on food security, productivity, dairy cow health and welfare. Some vaccines against mastitis are available although these are not widely licensed or have limited efficacy. For the purpose of improving mastitis control and of identifying T cell-associated determinants of protective immune responses, we evaluated the immune response and results upon challenge obtained after immunization against E. coli through different routes. Our objective is to favor a Th1/Th17 cell-mediated immunity to improve neutrophil recruitment and clearance of bacterial infections.

Materials and Methods: Holstein heifers were bred together from the age of 14 months until the end of the experiment. Two groups of six heifers each received one intramuscular injection of heat-killed E. coli P4 bacteria (2×10^9 colony-forming units, CFU) in Montanide™ adjuvant three months before calving. Two months later, a boost injection with the same preparation was done by intramuscular (IM) route in the IM immunized group, while the other group (IMM immunized group) received a diathelic injection of ultracentrifugated E. coli P4 culture supernatant (50 μ g proteins). Adjuvant only was administered to six control cows by the IM route. Approximately 50 days after parturition, all cattle were challenged with live P4 bacteria (10^3 CFU in total) through the teat canal of one healthy quarter (somatic cell count SCC<50,000 cells/mL). Clinical evaluation, in addition to laboratory testing, was performed during two weeks upon inoculation.

Results: Results showed strong antibody and cell-associated immune responses in the blood of immunized cattle, indicating that the immunization resulted in Th1/17 priming. Upon homologous challenge, clinical signs at the acute phase (12 hours pi) are less severe in the immunized cattle compared to the controls (3.0 vs 5.2 AU, Two-way ANOVA, p-value<0.05); IMM immunized cattle recover more rapidly thereafter (Cumulative clinical score, Mann-Whitney test, p<0.05). The reduction of milk production was significantly lower in the immunized groups (Two-way ANOVA, p<0.001), indicating that immunization was successful at reducing milk losses. No difference of local inflammation

was noticed in immunized cows, but the mammary inflammation in the IM immunized group tended to persist longer than in the control cows. Leucodepletion in the immunized groups was also significantly lower. Furthermore, the time required for bacterial clearance was half in immunized cows compared to the controls, although SCC were not significantly different amongst the groups over the mastitis course.

Conclusions: These results indicate that priming of Th1/Th17 immunity against *E. coli* improves significantly the conditions against clinical mastitis associated with this pathogen. Moreover, afforded protection is improved when specific cells are recruited locally through a mammary recall. Further studies are needed to determine mechanisms affording protection against Gram-negative mastitis, and the advantages and mechanisms of locally-administered vaccines over systemic immunization.

Immunology/Vaccinology

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Effect of injectable trace minerals on the immune response to bacterial antigens after administration of an attenuated-live bacterin in dairy calves

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Objectives: In a previous report we showed that injectable trace minerals (ITM) improved the humoral and cell mediated immune (CMI) responses to viruses involved in the bovine respiratory disease complex, following administration of a modified-live virus (MLV) vaccine in dairy calves (Palomares et al., 2015; AABP conference). The objective of this study was to evaluate the effect of an ITM supplement containing zinc, manganese, selenium, and copper on the humoral and cell mediated immune (CMI) responses to individual vaccine antigens in dairy calves receiving an attenuated-live bacterin containing *Pasteurella multocida* and *Mannheimia haemolytica*.

Materials and Methods: A total of thirty dairy calves (3 months of age) were administered 2 mL of an attenuated-live *M. haemolytica* and *P. multocida* bacterin (Once PMH®, Merck Animal Health) subcutaneously (SQ). Calves also received 2 mL of a 5-way MLV vaccine containing BHV1, BVDV1 & 2, BRSV, PI3V (Express 5®, Boehringer Ingelheim, Vetmedica), and were randomly assigned to 1 of 2 groups: (1) Administration of ITM SQ (1 mL/100 lb BW, ITM; Multimin 90, Fort Collins, CO; n = 15) or (2) Injection of sterile saline SQ (2 mL, Control; n = 15). Administration of ITM provided 15, 60, 10 and 5 mg/mL of Cu, Zn, Mn and Se. Three weeks after initial vaccination, calves received a booster of 2 mL of the attenuated-live bacterin and 2 mL of the 5-way MLV vaccine SQ. Concurrently with the vaccine booster, a second administration of injectable trace minerals or sterile saline SQ was given to calves in ITM and control group, respectively. Blood samples were collected into tubes with and without acid citrate dextrose to obtain whole blood and serum respectively on days 0, 7, 14, 21, 28, 42, 56, and 90 relative to prime vaccination for antibody titer determination, antigen-induced in vitro IFN-γ production by peripheral blood mononuclear cell (PBMC), and antigen-induced PBMC proliferation. Statistical analysis was performed

using the Statistical Analysis System (SAS®). Non-parametric analysis was applied using Mann-Whitney U and Friedman tests for comparisons between treatment groups and over time, respectively. For all analyses values of P < 0.05 were considered significant.

Results: A strong humoral immune response to *M. haemolytica* or *P. multocida* was not observed after the primary vaccination. However, both the ITM and control group had a significantly increased antibody response to both antigens after booster vaccination. Between days 21 and 56, the calves that received ITM had a higher antibody titers (and higher rate of increase) to *M. haemolytica* compared to the control group. Significant differences were not observed in the antibody titers to *P. multocida* between groups. Only calves treated with ITM showed an augmented PBMC proliferation response upon *P. multocida* stimulation on day 21 after prime vaccination compared to day 0 (P =0.03). On the other hand, control calves did not show any significant change in CMI to *P. multocida* over time. Significant differences were not found in the production of IFN-γ by PBMC upon stimulation with *P. multocida* between calves treated or not with ITM concurrently with bacterin administration.

Conclusions: In conclusion, administration of ITM concurrently with vaccination in dairy calves resulted in increased fold change in antibody titer to *M. haemolytica* (between days 21 and 56 after primary vaccination) compared to the control group and an augmented PBMC proliferation upon stimulation with *P. multocida* on day 21 compared to day 0. ITM might represent a promising tool to enhance the immune response to attenuated-live bacterin in an attempt to prevent respiratory disease in cattle.

Immunology/Vaccinology

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Protection against bovine respiratory syncytial virus provided by maternally derived antibodies from cows immunized with an inactivated vaccine

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Objectives: Our study aimed to assess the degree of clinical and virological protection against BRSV conferred to calves by maternally derived antibodies (MDA) contained in the colostrum of cows immunized with an inactivated BRSV-PI3-Mannheimia haemolytica vaccine (Bovilis® Bovigrip, MSD Animal Health) during late gestation.

Materials and Methods: Thirty-two dairy cows, BRSV-seronegative or with low BRSV-antibody titers, were separated into 2 groups. Sixteen cows received two doses of an inactivated BRSV-PI3-M. haemolytica vaccine (Bovilis® Bovigrip, MSD Animal Health), 8 and 4 weeks before their expecting calving dates, whereas the other 16 cows were not vaccinated. Colostrum from each cow was collected at calving, tested for BRSV-antibodies (ELISA and seroneutralisation (SN)), and stored at -20°C. Two months later, 28 neonate dairy calves were immediately separated from their dam after birth and fed colostrum (10% of body weight within the first 6 hours) either from the vaccinated (n=14, vaccine group) or from the non-vaccinated cows (n=14, control group). Quality of the passive transfer of the BRSV-specific MDA was assessed by ELISA and SN at 1, 7, 14 and 21 days after birth. At 22 ± 3 days after birth (D0), all calves were inoculated by the intranasal and intratracheal routes with

the BRSV 3761 strain (2×10^5 TCID₅₀/calf). Clinical examination was done daily from D-3 to D15. Nasal swabs were collected every 48 h from D-2 to D16, to monitor BRSV excretion by RT-qPCR (Taqvet BRSV, LifeTechnologies). Bronchoalveolar lavages (BAL) were performed at D0, D3, D6, D10 and D16 (5 calves/group) to quantify BRSV loads in the lower respiratory tract (RT-qPCR) and to study the host response by transcriptomic analysis of 60 bovine genes involved in inflammatory response (real time PCR Fluidigm technology). Calves were euthanized at D7 (5 calves/group) and D16 (17 calves) to assess gross and histological lung lesions.

Results: A strong BRSV antibody response was detected in the vaccinated cows by ELISA and SN although the increase of SN antibodies was obvious only after the second immunization. This resulted in high titres of BRSV ELISA and, to a lesser extent, SN antibodies in the colostrum of immunized cows and therefore in calves fed these colostrums. On the other hand, calves from the control group remained seronegative until the challenge. After the challenge, all calves from the control group showed clinical signs of respiratory disease. In contrast, 35.7 % (5/14) of calves of the vaccine group remained healthy and none had severe respiratory clinical signs. The mean clinical scores from D7 to D11 and the accumulative clinical score were significantly different between the two groups. Interestingly, approximately at the same period (D6 to D16), the quantities of BRSV-RNA detected in the BAL from calves of the vaccine group were lower than in the control group, suggesting that MDA partially blocked the BRSV replication in the lungs. On the other hand, BRSV-RNAs were detected in nasal swabs of all infected calves from D2 to D15 (peak at D6) with no significant differences between the two groups, suggesting that MDA didn't interfere with virus replication in nasal cavities. Gross and histological lung lesions in calves of the vaccine group tended to be less extensive and less severe at D16 than those of the control group but the differences were not significant. Finally, differences were observed between the two groups in the transcription levels of major inflammatory bovine genes.

Conclusions: MDA of cows vaccinated with Bovilis® Bovigrip conferred a partial but significant clinical protection to calves during the first 3 weeks of life, especially against severe BRSV disease. However, the calves fed the colostrum of vaccinated cows were not protected against BRSV replication in their nasal cavities. These results suggest that vaccination of BRSV-seronegative pregnant cows may be a strategy to reduce clinical consequences of BRSV infection at a herd-level, provided that a colostrum management program allowing an effective passive transfer of immunity in calves is implemented.

Immunology/Vaccinology

P02-002-144

Acute phase protein response in the calves administered with experimental *M. bovis* vaccine

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Objectives: *Mycoplasma bovis* is considered as a causative agent of different cattle disorders such as bronchopneumonia, arthritis, mastitis, keratoconjunctivitis, otitis and reproductive problems. A high resistance of majority isolates of the bacteria to veterinary-use antibiotics complicates the effective control of *M. bovis* infection. Moreover, there is still a lack of commercial vaccines in Europe, whereas data about such vaccines available in the United States is limited. The aim of the study was to

evaluate the acute phase protein response in the calves administered with experimental *M. bovis* vaccine.

Materials and Methods: Twelve clinically healthy female calves were used in the study. The calves were divided into two groups of six calves: experimental and control. The experimental calves were administered subcutaneously with the inactivated experimental vaccine composed of the Polish field *M. bovis* strain and a combination of saponin (Sigma, Poole) and Lydium-KLP™ (Nika, Health Products). Control calves were injected with sterile phosphate buffered saline by the same route. The blood samples were collected on days 0, 1-7, and then in 7 day intervals up to the end of the study at the 84th day of observation. Serum concentrations of selected acute phase proteins such as haptoglobin (Hp) and serum amyloid A (SAA) were determined using two different ELISA kits manufactured by Tridelta Development Ltd.

Results: In response to the experimental vaccine administration a distinct increase in the Hp concentration was observed at days one and two when compared to the control. During other days of observation the Hp values fell to the nought. On the other hand the calf vaccination caused above a six-times increase in the SAA concentration at day one in a comparison with the control. Distinct increased values of this parameter were registered up to day 6. However a renewed visible rise in the SAA concentration was observed at days 49, 56 and 84 post vaccination. During other days of observation the SAA concentration was close to the control values or slightly increased.

Conclusions: The calf administration of the experimental vaccine composed of the Polish field strain of *M. bovis* combined with saponin and Lydium-KLP™ caused a stimulation of acute phase response manifested with a significant increase in the concentration of Hp and SAA, indicative acute phase proteins for cattle.

Immunology/Vaccinology

P02-002-145

Subcutaneous ischiorectal fossa injection: serological, colostrum immunity and tissue reactivity in rota, corona and *E. coli* F5 (K99) vaccinated cows

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Objectives: Most labels in production animals indicate vaccination in the anterior third of the neck. In New Zealand (NZ) dairy, access to the neck is impractical and dangerous. Alternative injection sites meeting meat processing requirements is needed. Objective of this multi-site, parallel-group randomised non-inferiority study was to compare the immune response, measured by anti-rotaviral antibodies, and injection site reactivity, between cows vaccinated i.m. in the anterior third of the neck (IM) with cows injected s.c. in the ischiorectal fossa (IRF) using an oil emulsion vaccine containing inactivated rotavirus, corona virus and *E. coli* F5 (K99).

Materials and Methods: 120 healthy Friesian, Jersey and Friesian x Jersey cows, from two seasonally (spring) calving commercial dairy farms in the North Island of NZ, vaccinated with Rotavec Corona® (MSD Animal Health, NZ) in the previous calving season, were enrolled. On each farm, half the cows were randomly assigned to either the IM or IRF injection site and were grazed together on ryegrass/clover pasture until they calved. Rotaviral titres were determined in sera on days 0, 7, and 21 and immediately post-calving in sera and colostrum. Rotaviral

specific antibodies were quantified by virus neutralisation using a bovine rotavirus isolate (molecular typed as G6P5). Injection sites were scored on Days 0, 7, and 21. The primary outcome, colostral rotaviral antibody geometric mean ratio (GMR) of the IRF and intramuscular group, and its lower one-sided 95% CI, was estimated using linear regression with treatment group as the predictor, conditioned on variables considered a priori to be influential (i.e. farm, breed, age, and pre-vaccination serum titre). Model assumptions were evaluated by examination of residuals and generation of Cook's D values. Sensitivity analyses were conducted by removal of potential influential observations. Non-inferiority was declared if the lower limit of the 1-sided 95%CI of the GMR was above the non-inferiority margin 0.63. This margin corresponds to a difference of about two thirds of a twofold dilution in a serial dilution assay (USDA-vet service memorandum, 2007).

Results: The distribution, and geometric mean titre (GMT), of serum and colostral rotaviral antibody titres of cows vaccinated subcutaneously in the IRF was very similar to that of cows injected by the standard IM route. The lower bound of the GMR was 0.68, demonstrating statistically significant non-inferiority. No systemic adverse events were associated with either vaccination site. Thirteen of the fifteen largest lesions were observed in cattle vaccinated in the neck; but there was no evidence that the median volume was different between the different injection sites.

Conclusions: Vaccination with an oil adjuvanted, inactivated rotavirus, corona virus and E.coli F5 (K99) subcutaneously in the IRF is as effective as intramuscularly injection in the neck. This gives NZ vets the confidence to prescribe an alternative injection site and hence reduces the risk to farmers and themselves of injury during routine vaccination.

Immunology/Vaccinology

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Does BVD vaccination of recently weaned pasture-fed beef cattle prevent weight loss when exposed to persistently infected cattle?

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Objectives: Bovine Viral Diarrhoea virus (BVDv) commonly infects New Zealand (NZ) cattle and can cause significant production loss; especially reproductive failure. Although not considered as important, ill-thrift associated with transient infection may affect growth. The primary objective of this study was to compare the growth rate of vaccinated vs. non-vaccinated calves following exposure to persistently infected (PI) cattle.

Materials and Methods: The study population, in this single-site, randomised parallel group challenge field trial, was 80 weaned, mixed-gender, spring-born (August, 2013) Angus-x cattle from a BVD seronegative herd on a farm in the southern Wairarapa (North Island, NZ). Assuming a standard deviation (SD) of 100g/day (and alpha 0.05 and beta 0.80) the minimum detectable difference in average daily gain (ADG) was estimated as 63g/day. In May 2014 the cattle were randomised (blocked by weight and gender), into a non-vaccinated control or a vaccinated group (n=40/group). Calves were vaccinated with an inactivated Type I BVD vaccine (Bovilis® BVD, MSD Animal Health, NZ) on days 0 (V1) and 28 (V2). Six PI (BVD Type I) calves were introduced 14 days after V2 (day 42); they were initially yarded with the mob for 4 hours, and then remained within the mob for the remainder (70 days) of the study. The cattle were run as one mob on a brassica

crop with access to a run-off pasture and ad-lib water and meadow hay. A viral neutralisation test (VNT) was used to measure BVDv specific antibody on days 0, 42, 56, 70, and 84. The calves were weighed days 0, 42, 56, 70, 84, 98 and 112. ADG between PI introduction and day 98 was modelled using linear regression with the fixed effect of vaccination adjusted for baseline weight and gender (NB: data from day 112 was excluded due to a radical change in diet 24 hours prior to being weighed).

Results: At enrolment, mean weight was 218 vs 230kg for females and males resp., similar for the 2 treatment groups, and all calves were BVDv antibody negative (titres <1:2). All vaccinated cattle had titres 14 d after their 2nd vaccination, (1:16 to 1:512) with a median IQR of 1:64. Within 14 d of introduction of the PIs, 44% (17/39) of the non-vaccinated cattle seroconverted. At day 70 all had seroconverted. Assuming a ≥ 4x increase was an amnestic response, 15% (6/39), 67% (26/39), and 97% (38/39) responded < 14, 28 and 42 d resp., of PI introduction. The difference (%) in serological response of groups (14 d risk difference (RD) is 28% [95%CI 9 to 47%, P_{chi2} = 0.0063] and the 28 d RD is 33% [19 to 48%, P_{chi2} = 0.0001]). Given exposure risk to PIs was assumed to be equal for all, differences suggest a different immunological response. Moreover, the geometric mean titre [95%CI] in the vaccinated cattle (34,000, [24,300 to 47,800]) was 15.2x greater ([7.4 to 24.4], P = <0.0005) than the non-vaccinated (2,200, [1,600 to 3,200]) at the last blood test 42 d after PI introduction. This shows a marked amnestic response in the vaccinated cattle. After vaccination and prior to challenge the 2 groups had equal ADG indicating no adverse event on growth (Control, 980 [916 to 1044] g/day vs vaccinated, 1011 [949 to 1072]g/day; a difference of 30 [-57 to 118]g/day, P = 0.49). Vaccinated cattle, adjusted for gender and starting weigh, had on average a 56.8g/d greater ADG over the 56 d after the PI cattle introduction. This difference was almost significant (95%CI -3.0 to 116.6g/day, P= 0.062).

Conclusions: BVDv rapidly spread through the study population. Vaccinated cattle showed a marked difference in the immune response compared with non-vaccinated cattle. The 56.8g/day greater ADG of the vaccinated calves, is suggestive of a protective vaccine effect; however given the greater variability in the study population than was anticipated (i.e. SD of 133g/day vs. assumed 100g/day) the study had insufficient power to discriminate this difference with 95 % confidence. This small study indicates transient BVDv infection may affect growth in pasture-fed cattle and vaccination may reduce this effect, however further studies are required.

Immunology/Vaccinology

P02-002-147

Puerperal and milk shedding of C. burnetii are associated with different immune profiles

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Objectives: C. burnetii (Cb) causes Q fever in humans and Coxiellosis in animals. Serological diagnosis in human medicine relies on two antigens: Phase I (PhI) and Phase II (PhII). PhII-antibodies are detected early after infection, high-level PhI-titers are indicating a chronic course of infection. Regarding dairy cows repeated detection of Cb in milk was associated with increased PhI-titers, too. In this study we assessed immune profiles (phase-specific antibody, IFN-γ-responses) of cows with different Cb-shedding histories before and after vaccination with an inactivated Cb vaccine (Coxevac).

Materials and Methods: Blood samples were tested for phase-specific antibodies (ELISA) and Cb-specific interferon- γ (IFN- γ) reactivity by an IFN- γ -recall assay. Cows in an Cb-infected herd were repeatedly tested for milk (MS) and puerperal shedding (PS) by quantitative PCR over a period of 2 ½ years prior to vaccination. Subsequently, cows were allocated to the following groups: PS+/MS+ (n=16), PS-/MS+ (n=36), PS+/MS- (n=17) and non-shedders (NonS; n=37). A group of primiparous cows that had been vaccinated prior to first breeding (Coxevac) was included as a 5th group (H/Vacc; n=11). Shedding within the last 6 months or more than 6 months prior to testing was included as an additional variable. After vaccination the following groups were compared: cows shedding Cb in milk (MS+/PS-/+), shedding Cb at calving (PS+/MS-), non-shedders with antibodies (NonS/Ab+) and seronegative non-shedders/IFN- γ -negative (neg).

Results: Prior to vaccination groups PS+/MS+ and PS-/MS+ showed strong PhI- and PhII-titers. PhII-titers and low/absent PhI-titers were observed in groups NonS and PS+/MS-. An inverse reactivity was detected by IFN- γ -recall assay i.e. higher IFN- γ -reactivity was found in PS+/MS- compared to PS+/MS+ and PS-/MS+. If the last shedding occurred more than 6 months ago a shift in reactivity was observed: IFN- γ -reactivity (PS+/MS+; PS-/MS+, PS+/MS-) was increased and PhI-titers (PS-/MS+) decreased.

After vaccination both PhI and PhII-titers increased except for the negative group and H/Vacc, in these animals only PhII-titers were detected. Groups PS+/MS- and NonS/ab+ showed a strong IFN- γ -response already after first vaccination. It might be explained by a booster of preexisting immunity. In contrast, IFN- γ -response in group MS+/PS-/+ developed slowly and even after 2nd vaccination some animals failed to develop an IFN- γ -response.

Conclusions: Puerperal shedding of Cb was associated with a favorable immune response before and after vaccination. Shedding of Cb in milk - irrespective of puerperal shedding - was associated in some animals with an inappropriate immune response even after vaccination. This failure might be related to a chronic course of infection. Currently, we are using PhI-titers >100 as a suitable marker of chronic Cb-shedding in milk, which is confirmed by qPCR.

PhII-titers in absence of PhI-titers are regarded as an indicator of acute infection in humans. This is principally true for cattle, too, but it was also observed after resolution of infection or vaccination.

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Immunology/Vaccinology

P02-002-148

Impact of oral meloxicam and transport on CMI and humoral immunity in feedlot steers receiving a MLV BVDV booster vaccination at arrival

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Objectives: The objective of this study was to investigate the impact of oral meloxicam (MEL) and long-distance transportation on cell-mediated immunity (CMI) in preconditioned steers receiving a booster vaccination on arrival. We hypothesized that steers treated with MEL at 1 mg/kg body weight 6 hours before night-time transport would be

less immunocompromised on arrival (day 0) and after 7 days, and that CMI following vaccination with a modified live bovine viral diarrhoea virus (BVDV) recall antigen would be increased.

Materials and Methods: Brahman crossbreed steers, 13 to 17 months of age (n=87), were randomly assigned to one of four treatment groups: MEL, transported (MTR) (n=22), MEL, non-transported (MNT) (n=22), lactose placebo, transported (CTR) (n=21), and lactose placebo, non-transported (CNT) (n=22). MTR and CTR steers were transported for approximately 16 hours non-stop on a truck from Mississippi to Iowa (approximately 1,300 km), whereas steers in the MNT and CNT groups remained in Mississippi as non-transported controls. Body weight was measured and jugular blood was collected at -1, 0, and 7 days from all steers at the same time, regardless of location. Multi-parameter flow cytometry (MP-FCM) was used to identify T-cell subsets and detect the expression of three activation markers (CD25 [interleukin (IL)-2 receptor], intracellular interferon-gamma [IFN γ], and IL-4) after *in vitro* stimulation with BVDV recall antigen. Plasma cortisol concentration was measured at days -1, 0, and 7 as a marker of transport-associated stress. Serum antibody titer to BVDV was assessed on day -1 and day 7 post-booster vaccination. Whole-blood samples were analyzed using MP-FCM on days 0 and 7. Results were log transformed and analyzed using a mixed effects model.

Results: Compared with non-transported controls, transport led to an increase in BVDV-induced expression of CD25, IFN γ , and IL-4 in CD4⁺, CD8⁺, and $\gamma\delta$ T-cell subsets (P<0.05). MEL treatment mitigated the transportation-associated increase in CD25 expression by peripheral blood mononuclear cells (PBMCs), CD4⁺, and $\gamma\delta$ T cells. CMI outputs for the MTR group were less than those of the CTR group (P<0.05); however, the MTR and NT groups did not differ (P>0.10). A treatment/transport interaction was noted for the increase in IL-4 expression by CD8⁺ T cells after transport, with a significant difference between the CTR and MTR groups at day 7.

Conclusions: In conclusion, the use of oral MEL prior to transport may have beneficial effects, but further research is needed to validate the effect of MEL treatment on specific T-cell subsets in transported cattle.

Immunology/Vaccinology

P02-002-149

IL-1 β overcomes the deficiency in IFN- γ production in calves

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Objectives: Vaccine development has shifted from whole-microorganism based preparations towards subunit-based vaccines which although safer are less immunogenic. A key factor in the efficacy of vaccines is the age of vaccination but the importance of age for adjuvanticity has not been fully resolved. While adjuvant research has traditionally focused on humans and mice, there is a growing demand for new and improved veterinary vaccines. This is particularly important with regard to increasing food production and preventing zoonotic diseases. Therefore, we aimed to address the mode of action and importance of age in adjuvant activation of bovine immunity.

Materials and Methods: Inflammasome and T-cell responses were observed in 5 Friesian calves over a period of 8 months. Each month, bovine peripheral blood mononuclear cells (PBMCs) were isolated

and treated with various doses of ConA, a T-cell mitogen capable of driving T-cell proliferation. Additionally, PBMCs were also treated with a combination of ConA and recombinant IL-1 β . To analyse inflammasome responses in calves from 3-10 months of age, bovine PBMCs were primed with LPS before being treated with an inflammasome stimulating adjuvant, named alum. Following stimulation, supernatants were harvested and tested for cytokines IFN- γ , IL-17, IL-4 and IL-1 β .

Results: While bovine PBMCs from 4 month old calves showed robust secretion of Th2 and Th17-associated cytokines in response to T-cell mitogen stimulation, the ability to produce the Th1-associated cytokine IFN- γ remained subdued until 6 months of age. Interestingly, IL-1 β enhanced secretion of IFN- γ in young calves. Similarly, IL-17 expression was increased in response to IL-1 β , while no enhancement of the Th2 associated cytokine IL-4 was observed. This suggests the potential of IL-1 promoting adjuvants as drivers of bovine Th1 immunity. Having shown that IL-1 β is an important mediator of Th1 and Th17 responses in calves, IL-1 β secretion was assessed over a range of ages. PBMCs from young animals (3-4 months) responded strongly to inflammasome activating adjuvants indicating that there is no age dependent defect in inflammasome activation and further suggesting the potential of adjuvants to drive this pathway as a means to enhance Th1 and Th17 responses in cattle.

Conclusions: This study confirms the selective defect in IFN- γ production by calf PBMCs but for the first time identifies different kinetics with regard to IL-17 secretion. Furthermore it suggests the potential of driving IL-1 β secretion by innate cells through vaccine-adjuvant formulations as a means to boost both IFN- γ and IL-17 secretion.

Immunology/Vaccinology

P02-002-150

Efficacy and safety of Zelnote®, a novel DNA Immunostimulant, in cattle

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Objectives: Bovine respiratory disease (BRD) negatively impacts the beef and dairy industries. Leveraging the innate immune system may improve the health of cattle at risk for BRD. The objectives of this series of studies were to evaluate the efficacy (Studies 1 and 2) and safety (Studies 3 and 4) of a novel DNA Immunostimulant (Zelnote®).

Materials and Methods: The DNA Immunostimulant was administered concurrently with (Study 1) or 24 hours after (Study 2) a multi-drug resistant Mannheimia haemolytica (Mh) challenge. Holstein steers (3-4 months of age; N=32/treatment, Study 1; N=40/treatment, Study 2) received the DNA Immunostimulant or a negative control on Day 0 (Study 1) or Day 1 (Study 2). Calves were challenged (intratracheally) with 60 mL of Mh (107 CFUs/mL, Study 1; 108 CFUs/mL, Study 2) on Day 0 and clinically observed to Day 5. Lung scores were obtained at the time of premature death or after necropsy on Day 5.

Twenty healthy Holstein bulls, 3-4 months of age, were enrolled in Study 3. On Day 0, the DNA Immunostimulant and the negative control was administered on opposite sides of the neck, intramuscularly, in each calf (each calf served as its own control). Injection site palpation occurred on Days 0-7. All calves were clinically observed for either 21 (N=10)

or 28 (N=10) days and subsequently necropsied. Tissue sections were harvested from both sides of each calf's neck (corresponding to each site of injection) and evaluated for evidence of gross and histopathological changes. Tissue specimens were examined by a board-certified veterinary pathologist.

In Study 4, 612 healthy, post-weaned, commercial calves \geq 3 months, were enrolled and the DNA Immunostimulant was administered, intramuscularly, to each calf on Day 0. Injection sites were palpated on Day 3. All calves were clinically observed for 21 days and were eligible for commerce at study completion.

Results: In this Mh model, the DNA Immunostimulant was observed to mitigate lung pathology by 40.3% (95% confidence interval [CI]; 13.9%, 65.3%) and 36% (95% CI; 3%, 64%) in Studies 1 and 2, respectively, compared to the negative control. In Study 2, cattle administered the DNA Immunostimulant on Day 1 displayed a significant reduction in mortality ($p=0.0394$) compared to cattle receiving the negative control on Day 1.

In Study 3, the DNA Immunostimulant was shown to induce no adverse reactions, relative to the negative control, at the injection site (both grossly and histopathologically). In Study 4, no adverse events were observed among large populations in different field scenarios.

Conclusions: The DNA Immunostimulant demonstrated efficacy by mitigating lung lesions (Studies 1 and 2) and decreasing mortality (Study 2) in this Mh disease model. Studies 3 and 4 display that the DNA Immunostimulant is safe for use in cattle. The DNA Immunostimulant (Zelnote®) is labeled as an aid in the treatment of BRD when administered at the time of, or within 24 hours after, a perceived stressful event. A 21 day meat withdrawal has been established for this product.

Immunology/Vaccinology

P02-002-151

Change of blood morphophysiological parameters in calves during vaccination under influence of metabolism stimulator Catosal

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Objectives: This field study demonstrated the peculiarity of calves immunity formation, and changes in morphophysiological blood parameters during vaccination period under the influence of immunomodulating product.

Materials and Methods: 30 calves of 30-day age were divided by the principle of analogues into three groups 10 calves each group. The animals from Group 1 received on Days 0 and 14 inactivated viral-bacterial vaccine against respiratory syncytial infection, parainfluenza-3, virus diarrhoea and pasteurellosis BioBos Respi 4 («Bioveta», Czech Republic) and metabolism stimulator Catosal («Bayer HealthCare LLC», USA), which contains butafosfan and cyanocobalamin. The animals from the Group 2 were treated by vaccine on day 0 on day 14 of experiment. The calves from group 3 were untreated control.

Number of erythrocytes, leucocytes, haemoglobin, haematocrit were estimated before treatment on Day 0 and then on Days 14 and 30. Erythrocytes, leucocytes (including lymphocytes, monocytes, granulocytes), thrombocytes, haemoglobin, haematocrit, mean cell haemoglobin, mean cell haemoglobin concentration were estimated on day 30. Antibodies against respiratory syncytial infection virus level

was determined by EIA method, using diagnostic test-systems of the company IDEXX, CIVTEST on Days 14, 30, 43 and 63.

Results: On Day 30 the blood parameters were higher in the Group 1 than in Group 2 and Control: erythrocytes by 13% and 28,8% ($p<0,05$), haemoglobin by 15% and 23% ($p<0,01$), thrombocytes by 36,3% and 31,8% respectively ($p<0,05$).

There was registered the decrease of leucocyte count on Day 14 at revaccination in Group 2 and in control group by 24,4% and 32,6% comparing to Day 0. On Day 30 leucocyte level in both groups increased, however, it was still below initial range. In Group 1 the animals demonstrated leucocyte count increase within normal range during the whole observation period. On Day 30 leucocyte count in animals from Group 1 was 23% higher the initial amount ($p<0,01$).

The antibodies level in Group 1 was higher than in Group 2 during the whole period of observation: by 2.5 times on Day 14, by 1,9 times on Day 30, by 1,4 times on Day 43 and 1,7 times on Day 63 ($p<0,01$).

Conclusions: Catosal provided immunomodulating effect, securing stable high leucocytes level during the whole observation period, and stimulated nonspecific immune response, which was confirmed by higher level of leucocytes and thrombocytes.

Concomitant administration of vaccine and Catosal stimulated specific immune response by organism, accelerating antibodies production and providing for higher dynamics of antibodies titre increase.

Immunology/Vaccinology

P02-002-153

Vaccination of dairy cows in late gestation: effect on serum and colostrum antibody titers for BRSV and Mannheimia haemolytica.

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Objectives: Vaccination of calves against BRD is overall effective but, in some field situations, i.e. high infectious pressure, early infection in very young calves before active vaccination, this could not be enough to protect them against disease.

The objective of the study was to observe whether vaccination of cows during late gestation with a respiratory vaccine influences levels of serum and colostrum antibodies in the cow and in their calves after colostrum intake.

Materials and Methods: The fieldwork was conducted by local veterinarians during the fall and winter 2013-2014 in Brittany. Two hundred Holstein cows from 13 farms were randomly selected to either vaccination ($n=100$) with antigens of BRSV, PI3 and Mannheimia haemolytica (Bovilis® Bovipast, MSD Animal Health) or no vaccination ($n=100$). Cows were vaccinated according to label; the first dose was injected between 8-6 weeks before expected calving and the second dose between 4-2 weeks before expected calving. Levels of antibody titers were compared between cows in each group and between cows and their calves from both groups raised on the same farms. Seroneutralizing antibody levels of BRSV were measured by a VN test (ISAE). To determine M. haemolytica antibody levels, a validated quantitative ELISA (MSD Animal Health) was used.

Results: After different steps of selection in the database (elimination of couples mainly because of missing data), 189 mother-calf pairs were included in the analysis. One hundred mothers were vaccinated before calving and 89 were not vaccinated. The animals were followed in 13 different herds and in each herd the number of vaccinated and unvaccinated animals ranged from 6-27. The levels of BRSV and Mannheimia haemolytica antibodies measured at the first blood test before vaccination did not differ between vaccinated and non vaccinated cows ($p>0.05$). Following vaccination, specific neutralizing antibody titers of BRSV tended ($p=0.07$) to be higher in colostrum from vaccinated mothers compared to control cows. However these levels were significantly higher ($p=0.01$) in the serum of calves born from vaccinated mothers compared to those from non vaccinated mothers. After vaccination, significantly higher levels of M. haemolytica antibodies ($p<0.001$) were measured in serum of cows, in the colostrum of vaccinated dams and in the serum of calves born from vaccinated cows, always in comparison to the control group.

Conclusions: This field study confirmed that vaccination of cows in late gestation, with a commercial trivalent vaccine increased the levels of serum and colostrum antibodies against 2 major respiratory pathogens. Vaccination of late gestation cows in addition to vaccination of calves, may prevent BRD more effectively, especially in herds either with a high prevalence of BRD in young calves (<3 weeks of age) and/or with high infectious pressure on all types of cattle present in the farms. Further research is needed to evaluate the return on investment of such vaccination programme.

Immunology/Vaccinology

P02-002-154

Fimbriae type I and PME-CD of Moraxella bovis and Moraxella bovoculi as antigens for vaccines against infectious bovine keratoconjunctivitis

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Objectives: Infectious bovine keratoconjunctivitis (IBK) is an important ocular disease that affects cattle worldwide. It has become a serious economic and health problem. The main aetiological agents associated with IBK are Moraxella bovis and Moraxella bovoculi which have important genetic differences.

The aim of this study was to evaluate the presence and expression of type I fimbriae and outer membrane protein (OMP)-CD of M. bovis and M. bovoculi strains isolated from clinical cases of IBK in Uruguay, in order to identify conserved and highly immunogenic antigens as potential vaccine components.

Materials and Methods: A collection of clinical isolates of M. bovis and M. bovoculi, previously characterized in our laboratory was used to evaluate the presence and expression of I fimbriae and OMP-CD in strains recovered from IBK cases in Uruguay.

Fimbriae type I (fim09) and OMP-CD (omp-cd) genes were detected by partial PCR amplification with specific primers designed according to M. bovis sequences. All PCR products were confirmed after sequencing and BLAST analyses.

Twitching motility was evaluated by dropping grown cultures of Moraxella strains on brain heart infusion (BHI) agar plates at 37 °C. After 96 h of

incubation, growth halos were measured and twitching motility of each strain determined.

Expression and purification of OMP-CD was performed in vitro according to standard outer membrane protein procedures. Purified OMP-CD was identified by 2D gels (SDS-PAGE) and subsequent mass spectrometry analyses. Finally, immunogenicity of this protein was assessed by Western blot using sera and tears of healthy and IBK-affected animals.

Results: PCR amplification showed that fim09 gene was amplified in all *M. bovis* strains whereas it was not detected in any *M. bovoculi* strain. On the other hand, omp-cd was amplified within the whole collection of *M. bovis* and *M. bovoculi* strains. OMP-CD possess a 70% nucleotide similarity between species.

Twitching motility analysis, to evaluate fimbriae expression, was detected in strains from both species of *Moraxella*, and external halos with variable sizes were observed within the collection.

Regarding OMP-CD expression, 2D electrophoresis and mass spectrometry analyses evidenced that this protein was expressed in both *M. bovis* and *M. bovoculi* strains.

Finally, immunogenicity of OMP-CD was assessed by evaluating the presence of antibodies in natural infections of IBK with Western blot. It was seen that OMP-CD was highly immunogenic in tears and in sera from animals.

Conclusions: It was seen that fimbriae genes are distributed within *M. bovis* strains. The same primers did not amplify this fimbrial gene in *M. bovoculi* strains. This suggests that fimbrial genes significantly differ between both species. The incorporation of *M. bovis* and *M. bovoculi*, both together in vaccine formula, could generate better protection against IBK. On the other hand, OMP-CD was present in all *M. bovis* and *M. bovoculi* strains. This antigen was highly immunogenic as proved after analysis of specific antibodies. These results suggest that OMP-CD is a conserved antigen, that could be considered for new vaccine formulations.

Immunology/Vaccinology

P02-002-155

Control of an outbreak of bovine brucellosis in dairy herds. Vaccination Strategy

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Objectives: Bovine brucellosis is actually present in all countries of Latin America. Control of this disease is based in serology, vaccination and elimination of positive animals. Although we know this strategy for many years, severe outbreaks of brucellosis still are present in the Continent. Mayor flows are lack of the enforcement of some control programs, and also a weak vaccination strategy. Two effective vaccines are available, S19 (smooth) and S RB51 (rough). Our objective is to show a successful strategy which can be able to control an outbreak of bovine brucellosis

Materials and Methods: Two dairy farms where brucellosis was present were selected for doing this study Farm A: 2224 animals with the initial prevalence of 1.75%, Farm B: 510 animals with an initial prevalence of 3.1%. Strain 19 was applied to all female calves between 3 and 8 months of age in both farms.

Serology: Buffer Plate Antigen Test (BPAT), Complement Fixation (CF) and Fluorescent Polarization (FPA) were the test used for diagnostic of brucellosis. A test and slaughter program was implemented. Due to poor results with the program, RB51 vaccine was applied in both farms to all S19 vaccinated animals. Positive animals by serology were eliminated as detected. Fetus and milk were analyzed by bacteriology methods. Strict management of the herd was implemented in order to avoid contact between infected and non-infected animals

Results: Animals were bleed and those positives were eliminated from the herd. Abortions were collected and burned as soon they were found. Serology indicates a strong infection. *Brucella abortus* biovar 1 was isolated. After a year of initiated the test and slaughter strategy results were very poor. Abortions were present and many positive animals were eliminated. *Brucella abortus* biovar 1 was isolated. For this reason, RB51 was applied in both farms to the whole herd, including pregnant animals. In consequence, serology went down to 0 and no record of abortions was observed

Both tables indicate that after RB51 vaccine implementation, brucellosis was controlled and eliminated from both herds. Two tables will show the evolution of the results

Conclusions: We studied two dairy farms, where animals, who were S19 vaccinated as calves, have a brucellosis outbreak and a test and slaughter program was applied. However after 6 months the results were very poor and RB51 vaccine was massive applied. Conclusion, S19 and RB51 are both good vaccines, however the importance is to know how and when those immunogens should be use. The advantages of RB51 are that it can be used anytime without complicate diagnostic and it doesn't induce abortion in pregnant animals previously vaccinated as a calf, but more important it is an excellent tool to control bovine brucellosis as was demonstrated in this study.

Immunology/Vaccinology

P02-002-156

EFFICACY OF A COMMERCIAL VACCINE WITH *Histophilus somni* AND LEUKOTOXOID OF *Mannheimia haemolytica* (HIPRABOVIS® SOMNI/Lkt) UNDER FIELD CONDITIONS

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Objectives: The aim of this study was to confirm the efficacy of a commercial vaccine containing *Histophilus somni* and Leukotoxoid of *Mannheimia haemolytica* (HIPRABOVIS® SOMNI/Lkt) in the reduction of respiratory clinical signs and in the number of concomitant treatments under real field conditions.

Materials and Methods: The study was performed in a farm with a census of 450 calves of different ages. Fifty-five animals between 4 and 6 weeks were included in the trial. These animals were randomly distributed in two groups taking into account their body weight on the day of vaccination. Animals from the negative control group received a placebo consisting of a buffered PBS solution (n=22). Animals from the vaccinated group received a commercial vaccine containing *Histophilus somni* and Leukotoxoid of *Mannheimia haemolytica* (HIPRABOVIS® SOMNI/Lkt) (n=33). Both groups received their treatments following the same route (subcutaneous), dosage (2ml/animal) and frequency (two doses, 21 days between first and second dose). Respiratory clinical signs and concomitant treatments were registered from all the animals

included in the trial to assess the efficacy of the vaccine. The significance level for of the statistical tests (Chi-square) was set at $\alpha=0.05$ (5%).

Results: Animals vaccinated with the commercial vaccine containing *Histophilus somni* and Leukotoxoid of *Mannheimia haemolytica* (HIPRABOVIS® SOMNI/Lkt) showed significantly less clinical respiratory signs and received less concomitant treatments than the negative control group.

Clinical respiratory signs (nasal secretion, ocular secretion, conjunctivitis, cough, respiratory rate and mucous nasal lesion) were observed in a higher percentage in animals from the control group (45.45%) than in animals vaccinated with the commercial vaccine containing *Histophilus somni* and Leukotoxoid of *Mannheimia haemolytica* (HIPRABOVIS® SOMNI/Lkt) (12.12%). Statistical differences between groups were observed (Chi-square test, p-value 0.005).

Concomitant treatments were registered in both groups during the whole study. In the control group 54.55% of animals were treated and in the vaccinated group 12.12%. Statistical differences were observed between groups (Chi-square test, p-value <0.001).

Conclusions: Vaccination with the commercial vaccine containing *Histophilus somni* and Leukotoxoid of *Mannheimia haemolytica* (HIPRABOVIS® SOMNI/Lkt) reduced the number of animals showing respiratory clinical signs and the number of concomitant treatments used during the first three months after the entrance in the fattening farm.

Immunology/Vaccinology

P02-002-157

Characterization of immunological gene expression in the intestine of healthy calves.

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Objectives: Gastrointestinal (GI) disease is a significant cause of morbidity and economic loss in young livestock. Understanding the pathophysiological significance of each element of the gut health 'triad' (immune system, microbiome, epithelium) will be useful in designing novel strategies to improve GI health in growing animals. In this study, we characterized mucosal inflammatory gene expression (IGE) profiles at different biogeographical sites of healthy calf intestines during the first 3 weeks of life.

Materials and Methods: Twelve calves from second parity cows on a single commercial dairy were removed from their dams immediately after birth and fed clean, high quality colostrum. The calves were fed milk replacer and health-monitored daily. A subset of calves (n=3) were euthanized at days 1, 3, 7, and 21, and mucosal tissue samples were taken from the duodenum, distal jejunum, ileum, and colon. Quantitative real time PCR analysis was performed to quantify the expression of interleukin 10, toll-like receptor (TLR) 2, 4, and 10, and tumor necrosis factor- α genes.

Results: The calves were clinically normal, and showed excellent feed intake and growth rates over the course of the experiment. There was a correspondingly low level of IGE in all calves, at all intestinal locations, and at all time points. The ileal mucosa exhibited a significantly higher pattern of IGE than all other sites, and TLR 4 had a three-fold higher expression than all other genes (p<0.05). The other sites sampled had low levels of both pro-inflammatory and anti-inflammatory cytokines relative to mean levels of gene expression from all samples.

Conclusions: As expected, the IGE profile of the intestine of this carefully managed and healthy calf group was subdued. The higher background of inflammatory activation in ileal mucosa could possibly be due to the relative abundance of Peyer's patches in this region. In future studies, we will compare the IGE profiles with the intestinal microbial communities and epithelial function of sick and healthy calves. Gaining an understanding of the connection between the immune system and microbial populations could lead to management solutions to GI health and reduce the use of antimicrobials.

Immunology/Vaccinology

P02-002-158

EFFICACY OF A COMMERCIAL VACCINE CONTAINING *Histophilus somni* AND LEUKOTOXOID OF *Mannheimia haemolytica* IN YOUNG CALVES UNDER FIELD CONDITIONS

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Objectives: Since 2011 an action plan on the rational usage of antimicrobials has been implemented by the EU Commission (Cars 2014). Antibiotics are the main measure for the control of pneumonic problems in cattle, while vaccination could be an alternative. The aim of this study was to demonstrate the efficacy under field conditions of a commercial vaccine with *Histophilus somni* and Leukotoxoid of *Mannheimia haemolytica* (HIPRABOVIS® SOMNI/Lkt) for the reduction of pneumonic problems caused by *Mannheimia haemolytica* and *Histophilus somni* in cattle and for the reduction of the number of drug treatments.

Materials and Methods: One-hundred-and-thirty calves between one and two months of age, clinically healthy, were randomly assigned to group A (n=64) and group B (n=66). Animals in group A were vaccinated subcutaneously with a commercial vaccine containing *Histophilus somni* and Leukotoxoid of *Mannheimia haemolytica* (HIPRABOVIS® SOMNI/Lkt) according to the recommended administration programme. Animals in group B (non-vaccinated) received PBS following the same schedule as group A.

Upon entering, the animals followed a standard protocol of hydration, deworming and bovine respiratory syndrome prevention (IBRV, RSV, BVDV and PIV-3 vaccine).

The main variables observed, recorded and compared to the control group were: clinical signs, lung damage and number of drug treatments. Different statistical test were performed. The level of significance used was 95%.

Results: Animals in group A (vaccinated calves) presented significantly lower clinical respiratory signs, lung damage and drugs treatments than the group B (non-vaccinated).

1. Clinical Respiratory Signs

Clinical respiratory signs scored from 0 to 3 (cough, dyspnea, nasal discharge, apathy, anorexia, lung auscultation, conjunctivitis and rectal temperature) were significantly higher in the group B (43.9%) than in the group A (20.3%) (Mann-Whitney test; p<0.01). Moreover, the true incidence for control and vaccinated groups was 4.72 and 1.40 episodes per 1000 animals at day of risk respectively (Mann-Whitney test; p<0.00001)

2. Lung Damage

The mean percentage of pneumonic tissue in the group A was lower than that for the group B (20.4% vs 41.3% respectively). When comparing the number of calves showing a percentage of pneumonic tissue equal or higher than 50%, the results were significantly higher in group B than in group A (6 vs 1) (T-test, $p < 0.05$).

3. Number of drug treatments

The number of treated animals, relapses, treatments and doses of drugs were significantly higher in the control calves than in the vaccinated ones (Chi-square and Mann-Whitney test, $p < 0.001$). 20.3% of the animals were treated in the vaccinated group, whereas 59.1% were treated in the non-vaccinated group. A total of 26 treatments as opposed to 144 were applied to vaccinated and control calves respectively. The number of doses of antibiotics administered to the non-vaccinated group was 5 times superior to the vaccinated one. Also, the number of doses of NSAID was 57 times superior in the non-vaccinated group.

Conclusions: The results of this study show that vaccination with the commercial vaccine with *Histophilus somni* and Leukotoxoid of *Mannheimia haemolytica* (HIPRABOVIS® SOMNI/Lkt) in field conditions achieves the reduction of pneumonic problems in calves based on the significantly decreased of clinical respiratory signs and pneumonic lung lesions, as well as the number of antibiotic treatments. Therefore, this vaccine would potentially help to follow the guidelines of the EU Commission by reducing the use of antimicrobials and improving the holdings health status.

Immunology/Vaccinology

P02-002-159

Passive Immunity And Natural Infection To Virosis Involved In Bovine Respiratory Disease

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Objectives: The purpose of this research aims to evaluate the dynamics of specific neutralizing antibodies (Abs) for respiratory virosis and lymphocyte subpopulations in calves from birth to 240 days (d) of life.

Materials and Methods: Therefore, 19 calves negatives in serum neutralization (SN) and RT-PCR for BVDV were selected. Colostrum from cows vaccinated semiannually were selected by Brix index ($\geq 21\%$) and total protein ($9.6 \pm 1.6\%$), and stored at -20°C . Calves received a minimum amount of four liters of colostrum, divided into two feedings, in the first twelve hours after birth (a.b.). Among the 19 calves, five were randomly selected for monitoring the dynamics of neutralizing Abs and subpopulations of lymphocytes present in the blood front the natural challenges from 14 to 240d.

Results: Seroconversion was observed in 19 calves (100%) for all viruses after colostrum intake, displaying medians (Log_2) of 12.3, 9.0, 5.0 and 8.5 for BVDV, BoHV-1, BRSV and BPIV-3, respectively. BRSV (11.3-2.0) present oscillation of the Abs titer (Log_2) throughout the study: 3.0; 3.0; 4.0; 3.0; 0.0; 3.0; 0.0; 3.0; 3.0 to 14, 24, 74, 90, 120, 150, 180, 210 and 240 days of life. Median of the Abs titer (Log_2) demonstrated gradual decline for BVDV (12.8-3.3), BoHV-1 (10.0-3.3) and BPIV-3 (10.0-2.0). Despite this, the individual analysis of the Abs from the 5 calves showed an increase of the Abs titer for BRSV and

BoHV-1 between 90 to 120 d, and the BPIV-3 between 180 and 240 d. The half-life of Abs and time to seronegativity obtained were 36.2 ± 6.1 and 367.01 ± 68.7 d for BVDV; 50.7 ± 18.0 and 239.67 ± 66.88 d for BoHV-1; and 46.8 ± 21.1 and 303.36 ± 60.15 d for BPIV-3. B cells $\text{CD}21^+$ increased from 14 to 150d. Variations in T-cell proportions preceded the increase of the individual Abs titer. T helper lymphocytes showed slight increase between 44-74 days, and then, an abrupt reduction in its proportions between 90 to 150 days, and an increase in subsequent moments. $\text{CD}3^+\text{WC}1^+$ showed sharp reduction between 44-74 days followed by maximum peak between 74-90 days, with subsequent drop (120 d) and increase in subsequent moments. Only 2/5 calves demonstrated $\text{CD}3^+\text{CD}8^+$ response observing a peak between 120 to 150 d. Some calves showed decline of $\text{CD}25^+$ lymphocytes between 90-150 d. Bronchopneumonia diagnosis was confirmed by clinical examination in 4/5 calves between 90 and 120 days of age.

Conclusions: Passive immunity protected calves against natural infection after birth up to 74 days. Therefore, calves showed T cell response, specially $\text{CD}3^+\text{WC}1^+$, increase of specific antibody titers for BRSV and BoHV-1 and clinical findings to Bovine Respiratory Disease.

Immunology/Vaccinology

P02-002-160

Duration of positive PCR results for BVD, IBR, BRSV and PI3 following modified live viral systemic or intranasal vaccination in sero-negative calves

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Objectives: to determine of duration of positive PCR and cycle time result for BVD, BoHV-1 BRSV and PI3 on blood, nasal swabs and transtracheal washes following vmodified live viral accinates administered systemically or intransally in sero-negative calves.

Materials and Methods: Twenty- 7 month old, BoHV1, BVDV, PI3 and BRSV sero-negative and BVDV virus negative calves were enrolled in the study. Animals were randomly assigned to group 1 vaccinated with a modified live viral vaccine containing BVDV types 1 and 2, BoHV1 BRSV, and PI3 (BoviShield Gold 5, Zoetis, INC) administered SQ or modified live BoHV1, BRSV and PI3 (Inforce 3, Zoetis, INC) administered intranasal. Calves had samples collected for PCR analysis (virus presence and cycle times) on day of vaccination, 72 hours post vaccination, and weekly until all calves were PCR negative for the various viruses. Samples consisted of deep nasopharyngeal swabs, whole blood (BVDV vaccinated group only) and transtracheal washes. A multiplex PCR for BoHV -1, BVDV (BVDV vaccinated calves only) types 1 and 2, Bovine Respiratory Syncytial Virus, and Parainfluenza virus-1 was run and cycle times were recorded. Calves required two successive weekly negative tests to be considered negative.

Study Design:

Calves were tested several times to ensure disappearance of colostrum antibodies against BoHV-1, BRSV, BVD and PI3. When the calves were sero-negative, samples were collected for BVDV persistent infection determination.

On the day of vaccination, all calves had serum and whole blood drawn, deep nasal swabs collected and transtracheal washes performed. SN titers were again performed and PCR for the presence of all five viruses

was run on these samples. Following collection of the samples, the calves were administered the appropriate vaccine: Bovishield Gold 5 or Inforce 3

Results: Intranasal vaccination: By day three after vaccination, calves vaccinated with Inforce 3 became positive on deep nasal swabs for both BoHV-1 virus (10/10) and BRSV virus (5/10), on day 3 transtracheal washes 3/10 calves were BRSV virus positive. Both samples were negative for PI3 virus. BoHV1Cycle times were variable with several calves positive under 30 CT (IBR-26-32), BRSV cycle times were higher on day 3 (30-38). All calves were BoHV-1 PCR negative on both nasal swabs and transtracheal washes by day 21. All calves were BRSV PCR negative on both samples by day 35 post vaccination. As in previous studies two calves were negative for one week and then became positive one week later then remained negative for the remainder of the trial. One calf was PCR positive for a single day (day 7) for PI3 virus. All buffy coat samples were negative on day 0 and were not tested again since BVDV was not included in the Inforce vaccinated group.

Systemic vaccination: A maximum of 4 calves were BVD virus positive by 14 days after vaccination on nasal swabs. A fewer number were detected using TTW or buffy coats. All positives were extremely weak with cycle times above 35 before any virus was detected (table 2). A total of four calves (2asal swabs and 2 TTW) were positive for BoHV-1 Again high cycle times were seen with the lowest time being 32 cycles. As anticipated both TTW and Nasal swabs were negative on all days following vaccination for both BRSV and PI3. All ten calves were virus negative by 21 days after vaccination.

Conclusions: This study provides valuable information on the duration of positive PCR for the viruses contained in two popular cattle vaccines. Many laboratories have tried to use cycle times as a method for differentiating wild virus exposure from PCR positives from vaccination. While this may be accurate when assessing PCR after systemic vaccination, the variability and low cycle times in the calves vaccinated intranasally make this method unacceptable. Durations of virus shedding may be longer if the calves are highly stressed but it is unlikely that many calves will be PCR positive to any virus in the vaccines by thirty days after vaccination.

Immunology/Vaccinology

P02-002-161

Comparison of interferon and BoHV1 IgA levels in nasal secretions of dairy cattle vaccinated with Inforce 3 prior to calving or on day of calving

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Objectives: To compare the level of antibodies against BHV-1 and levels and duration of interferon release in nasal secretions of cows vaccinated pre-calving or on the day of calving with a modified live viral, intranasal vaccine

Materials and Methods: Thirty two (2 parity of higher) Holstein cows were allocated to either receive intranasal vaccination (modified live

BoHV-1, BRSV and PI3, Inforce 3, Zoetis INC) two weeks prior to their projected calving date, or within 24 hours after calving. Nasal secretions were collected twice at a 12-hour interval on the day prior to vaccination (d = 0) and at 2, 4, 7, 10, and 14 days post vaccination to assay for IFN-alpha, IFN-beta, IFN-gamma and IgA directed against BHV-1; all were measured by ELISA.

Results: BHV1-specific IgA in nasal secretions: All cows seroconverted post vaccination. Significant increases in BHV-1 nasal IgA were seen in both groups. By day 4 after vaccination, cows vaccinated day of calving had significantly higher antibody levels than cows vaccinated pre-calving. These differences continued throughout the trial however by day 14 cows vaccinated pre calving were approaching similar levels of BHV1 IgA.

Interferon

Cows vaccinated on the day of calving showed minimal Alpha interferon responses. Cows vaccinated pre-calving showed two increases in Alpha interferon over baseline values. The first increase in levels was seen at 7 days after vaccination and a second increase began at 10 days after vaccination. By 14 days after vaccination the Alpha interferon levels were still climbing.

Both groups of cows showed an increase in beta interferon after vaccination. Fresh cows had a higher early release of Beta Interferon. Dry cows increased by day 4 after vaccination but by day 7 the levels seen post vaccination were at the same levels in both groups. Beta interferon levels again increased at ten days post vaccination in both groups.

The highest interferon levels were seen with gamma interferon. Cows vaccinated on day of calving showed an initial increase in Gamma interferon seen by day 4 with a second and higher level seen on day 10. The concentration of Gamma interferon in cows vaccinated on day of calving were beginning to decline by day 14 after vaccination. Cows vaccinated pre calving showed no increase in Gamma or total interferon until day 10.

Conclusions: All vaccines had increase in BHV-1 nasal IgA. In spite of prior viral vaccination during that lactation, cows still responded to an intranasal vaccination. The higher responses in both IgA and interferon post vaccination in cows vaccinated the day of calving in comparison with cows vaccinated pre-calving is in contrast to previous published studies demonstrating severe immune suppression following calving in dairy cows. However, these studies measured systemic immune suppression not local immune system responses. Either vaccination time may be appropriate in order to obtain both immune modulation due to interferon and specific immunity.

Immunology/Vaccinology

P02-002-162

Comparison of Cytokine mRNA of Peripheral CD4+ CD8+ and gamma delta T cells between Healthy Holstein and Japanese Black Calves

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Objectives: Weak calf syndrome presents as a newborn calf that is weak, sickly or slow to rise, and it appears mainly on Japanese Black (JB) in Japan. The function of T cells in JB calves may be inferior compared

with the other species. The objective of this study was to clarify the difference of leukocyte population and these cells function between healthy JB and Hol calves. Differences in the cytokines expression of CD4⁺, CD8⁺ and $\gamma\delta$ T cells will facilitate a better understanding of the differential function of T cell subsets in the immune response between two species calves.

Materials and Methods: Healthy 24 JB and 24 Hol calves were obtained at 1 week (N=8), 1 month (N=8) and 3 month (N=8) of age, respectively, and peripheral blood mononuclear cells (PBMCs) were aseptically separated. PBMCs were plated at 5x10⁶ cell/mL concentration in RPMI-1640 culture medium, containing 10% heat-inactivated fetal calf serum (FCS). We stimulated PBMCs for 12 hours at 37°C, in 5% CO₂ with 10 mg/mL phytohemagglutinin (PHA; Sigma Chemical Co, St. Louis, MO, USA). PBMCs were used for separation to CD4⁺, CD8⁺, and WC1-N1⁺ $\gamma\delta$ T cells by magnetic cell sorting (MACS) after the incubation. Enriched CD4⁺, CD8⁺, and WC1⁺ $\gamma\delta$ T cells were washed with PBS, and resuspended in TRIzol reagent. The target DNA sequence was amplified by specifically designed primers for β -actin, IFN- γ , IL-2, IL-4 and IL-10. Real-time reverse transcription polymerase chain reaction (RT-PCR) was performed with SYBR Green Master Mix.

Results: There were statistic different levels of mRNA expression in WC1-N1⁺ T cell between two groups. Levels of cytokines expressions in the JB Group were lower than those in the Hol Group in this study, and all analyzed cytokines were significantly different at month 1, and there were significant low levels of IL-2, IL-10 and IFN- γ expression at month 3. Although levels of cytokines expressions had little to no difference in the JB Group among the each analyzed points, expressions of cytokines at week 1 were lower than those expression at month 1 and 3 in the Hol Group

Conclusions: Our data substantiated the deficiency of cytokines expression activity in $\gamma\delta$ T cell on the JB calves. Since cytokines mRNA expression reaction of in $\gamma\delta$ T cells of JB calves was lower, reduced this cell function could aggravate infectious diseases and may explain some of the immunologic abnormalities accompanied.

Immunology/Vaccinology

P02-002-163

Antigenicity differences between Japanese and European *Staphylococcus aureus* and *Escherichia coli* bovine mastitis isolates

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Objectives: The objective of this study was to analyse the reactivity of the antibodies induced by the STARTVAC[®] vaccine against *Staphylococcus aureus* and *Escherichia coli* bovine mastitis isolates from different areas of Japan and Europe.

Materials and Methods: In this study, the antigenicity of *S. aureus* Japanese isolates was evaluated by Western Blot using serum from a cow vaccinated with STARTVAC[®]. With this propose, cell wall-associated proteins from *S. aureus* were extracted, analysed in SDS-PAGE and immunodetected with the specific antiserum. In order to demonstrate that the antigenicity of the core lipopolysaccharide is constant in different isolates of *E. coli*, the reactivity of a specific monoclonal with several isolates of this bacterium was assessed. This was performed by exposing the monoclonal "E. coli J5 LPS PIERCE" to an indirect ELISA coated with different isolates of *E. coli*. In the same way, the ability of a serum from a

cow vaccinated with STARTVAC[®] to react against several *E. coli* isolates from different origins was studied also by an indirect ELISA.

Results: The *S. aureus* cell wall-associated proteins analysed by SDS-PAGE showed a pattern similar between the Japanese and European isolates. Moreover, this study demonstrated the antigenicity of the *S. aureus* isolates against the antibodies induced by the STARTVAC[®] vaccination. Concerning *E. coli*, all the Japanese and European isolates analysed by ELISA shared the same epitope in the lipid A of LPS recognised by a specific monoclonal antibody and, moreover, antibodies induced by STARTVAC[®] vaccination reacted against all the isolates.

Conclusions: In conclusion, this study indicates that *S. aureus* and *E. coli* bovine mastitis isolates from Japan and Europe, present common surface antigens recognized by antibodies induced with STARTVAC[®] vaccination.

Immunology/Vaccinology

P02-002-164

Viability of bronchoalveolar CD14⁺ cells on calves infected with *Mannheimia haemolytica*

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Objectives: *Mannheimia haemolytica* is a commensal bacteria that resides in the upper respiratory tract of bovines that plays a major role in Bovine Respiratory Disease Complex and it is major cause of the disease's evolution into a fibrinonecrotic pneumonia. This study used an experimental infection model to investigate the effect of *M. haemolytica* and the antimicrobial treatment on the viability of bronchoalveolar CD14⁺ cells.

Materials and Methods: The study inoculated *M. haemolytica* in 12 healthy Holstein calves, all of them at 6 months of age. CD14⁺ cell viability was evaluated in four distinct moments: M1: 2 days before intratracheal inoculation; M2: at the beginning of bronchopneumonia clinical signs; M3: 1 day after the end of antimicrobial treatment; M4: 1 week after the end of antimicrobial treatment. The calves were uniformly divided in 2 aleatory groups of 6 animals right after M2: Group 1 (G1): calves were treated with norfloxacin (5 mg/kg, SID for 7 days) and anti-inflammatory flunixin meglumine (2.2 mg/kg, SID for 3 days); Group 2 (G2): animals were treated only with norfloxacin.

Bronchoalveolar lavage (BAL) was performed by bronchoscopy using a flexible colonoscope (EC-250LP5, Fujinon[®], Saitama, Japan), attached to an image processor with a light source. The BAL was performed by squirting 80 mL of sterile saline through the colonoscope working channel and then collecting the fluid, which was centrifuged and the cellular pellet was resuspended in RPMI 1640 medium enriched with FBS 10%. BAL fluid samples tested for cell viability by employing the Annexin V-FITC (APOPTEST[™], Dako Cytomation, Finland, cat K2350) and propidium iodide. These procedures were conducted according to the flow cytometry techniques. The identification of CD14⁺ cells was based on their cytoplasmic granularities and mean fluorescence intensities following a two-step fluorescent immunolabeling protocol using primary anti-bovine monoclonal antibody (CD14) and secondary antibody coupled to long-wavelength fluorescent probes.

Results: The percentage of apoptotic and necrotic cells were analyzed in quadrants. The lower left quadrant represents viable cells, the upper left the apoptotic cells, the lower right the necrotic cells and the upper right cells that are both in late necrosis or secondary apoptosis.

The samples analyzed at the beginning of clinical signs of *M. haemolytica* infection (M2-between 15 and 18 h after inoculation) showed a lower percentage of apoptotic cells (2.13% in G1 and 1.12% in G2, $P=0.011$ and $P=0.004$, respectively) and a larger percentage of necrotic cells (70.35% in G1 and 78.55% in G2, $P<0.0001$ for both) when compared with M3 (3.29% in G1 and 5.93% in G2 of apoptotic cells, 28.55% in G1 and 27.00% in G2 of necrotic cells). This fact may be due to the large number of bacterial pathogens causing tissue damage, which in turn lead to necrotic cell death that amplify the inflammation signals. A different explanation for the increase of necrotic cells would be that *M. haemolytica* leukotoxin has the ability to induce apoptosis in bovine leukocytes, but in the presence of inflammatory cytokines, such as IL1 β and TNF α , these cells undergo necrosis. Other reason would be that infection or ischemia could induce necrosis, since they these processes involve cell death factors such as TNF α .

In order to combat the infection by the etiological agent, cells produce reactive oxygen species that help to eliminate the microorganism, but at the same time can cause tissue damage. Thus, not only cell apoptosis, but also phagocytosis of apoptotic corpuscles are important in the resolution of inflammation.

Conclusions: In conclusion, the experimental infection by *M. haemolytica* altered viability of CD14⁺ cells of BAL fluid. Additionally, it was possible to observe that the percentage of viable cells in the animals after antimicrobial therapy return to the normal values before experimental infection with *M. haemolytica*, and there was no observed benefit to combine the anti-inflammatory, since there was no difference in the apoptosis rates between G1 and G2 in BAL samples.

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Internal Medicine

P03-003-102

Efficacy and pharmacokinetics of intravenous famotidine in adult cattle

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Objectives: The objectives were to measure the pH of abomasal outflow fluid as an indicator of abomasal pH in adult cattle after administration of the H₂-receptor agonist famotidine as a single dose of 0.4 mg/kg IV or as three doses of 0.4 mg/kg IV every eight hours to mimic clinical application and secondly to describe the pharmacokinetic behavior of intravenous famotidine in adult cattle.

Materials and Methods: A prospective random two-way crossover study design was implemented using 4 healthy adult Angus steers previously fitted with duodenal cannulae located orad to the bile and pancreatic ducts. The steers were fed alfalfa hay ad-lib and whole corn and soybean hull meal pellets at 4 lbs/head 12 hours apart. On Day 1, steers were administered either 0.4 mg/kg famotidine or an equal volume of saline via jugular catheters. Whole blood and abomasal outflow samples were obtained at intervals for a 12-hour period. On Day 2, steers were administered either 0.4 mg/kg famotidine or saline IV at time 0, 8, and 16 hours. After a 24-hour washout period, the opposite treatments were administered and the study repeated. Whole blood and abomasal outflow samples were obtained at intervals for a 24-hour period. Abomasal outflow fluid was tested for pH using a commercial bench top pH meter within 5 minutes of sampling. Fluid pH was analysed using a mixed model in SAS 9.4 with treatment, sampling hour, and treatment*hour as fixed effects and steer and treatment period as random effects. Least square means were separated using least significant difference. Serum was separated and stored at -20° C until pharmacokinetic analysis. Serum famotidine analysis was performed using tandem liquid chromatography mass spectrometry and pharmacokinetic analysis was performed using commercial software.

Results: The treatment*sampling hour interaction affected abomasal outflow pH for both a single dose and multiple doses of famotidine (P < 0.001). A single dose of intravenous famotidine resulted in a significant increase of abomasal outflow pH as compared to the control group for 4 hours after administration (P < 0.05). When administered every 8 hours, the abomasal outflow pH of the famotidine treated group was greater than the control group for 4 hours after the first dose (P = 0.03), 2 hours after the second dose (P < 0.0001), and 1 hour after the third dose (P < 0.0001). Pharmacokinetic analysis demonstrated that famotidine had a terminal half-life of 3.33 (3.21 – 3.54; median and range) hours, a volume of distribution of 0.042 (0.014 – 1.89) L/kg and a clearance of 1.26 (0.625 – 11.5) mL/min/kg.

Conclusions: A single intravenous dose of famotidine administered at 0.4 mg/kg increases abomasal outflow pH of adult cattle for up to 4 hours compared with saline control. Administration every 8 hours to mimic a clinical therapeutic regime resulted in significantly higher abomasal outflow pH for 4 hours after the first dose; however, the effect seemed to decrease after each subsequent dose. Results of this study indicate that famotidine raises the abomasal pH and may be an effective treatment for abomasal ulceration in cattle. The efficacy of treatment may decrease over time or may require more frequent dosing to sustain elevated abomasal pH.

Internal Medicine

P03-003-103

The effect of milk replacer and different components of milk replacer in an intestinal loop model for haemorrhagic enteritis in calves.

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Objectives: Recently, an intestinal loop model was developed to induce and study Clostridium perfringens induced necrohaemorrhagic enteritis in calves. In this model co-inoculation of C. perfringens with commercial milk replacer (MR) had a strong predisposing effect on the development of necrosis in this model. MR is composed by a number of dairy-derived ingredients, and for economic reasons also vegetal-derived ingredients. The objective of the present study was to identify the predisposing component of MR.

Materials and Methods: An intestinal loop model was conducted as previously described, by injecting intestinal loops with C. perfringens culture. In the first experiment, different concentrations of MR (0%, 0.25%, 2.5%, 10% and 25%) were made by dissolving commercial MR in sterile isotonic saline. Every concentration was tested in 6 loops, in 2 calves. In each loop 20 mL of MR solution in combination with 20 mL of C. perfringens culture grown overnight was injected. In the second experiment, the predisposing effect of two commercial MR's (MR1 and MR2) either with and without skimmed milk powder were compared, with saline as a negative control. The experiment was repeated in 3 calves, with 5 loops of each condition in each calf. Each loop was injected with 20 mL of bacterial culture and 20 ml of a 25% MR solution. In the last experiment, the predisposing effect of different components of commercial MR was tested. Loops were randomly divided over 3 calves, and 3 loops per ingredient were injected with bacterial culture in combination with a 25% solution of each of the following ingredients of MR: skimmed milk powder, casein, lactose, calcium carbonate, soy protein concentrate, potato protein concentrate, whey powder, whey permeate and whey protein concentrate.

Results: In the first experiment, necrosis could be induced in 40±0.23% of the loops injected with a 25% MR solution in combination with C. perfringens culture. In none of the loops injected with lower concentrations of MR necrosis was observed. There was no significant difference between the two tested MR's. However, significantly more loops inoculated with culture and milk replacer showed necrosis compared to loops inoculated with culture and saline. Loops inoculated with sterile medium and MR showed negligible number of loops with necrosis (0±0% for MR1, 0.03±0.05% for MR2). Loops inoculated with culture and both MR's showed similar proportion of loops with necrosis (respectively 0.67±0.19% for MR1, 0.65±0.28% for MR2), and only 0.27±0.19% of the loops inoculated with culture and saline showed necrosis.

In the final experiment, the most loops with necrosis could be induced by injecting culture in combination with MR (67±52%) and skimmed milk powder (60±23%), followed by whey permeate, casein, whey protein concentrate, whey powder and lactose (40±52%, 20±45%, 20±42%, 10±32% and 10±32% respectively). None of the loops injected with one of the other components showed necrosis on histological examination. There were significantly more loops with necrosis in the loops inoculated with culture and MR and culture with skimmed milk powder compared to all other components, except whey permeate.

Conclusions: The predisposing effect of MR on haemorrhagic enteritis in calves is concentration-dependent. In high concentration, different commercial MR's and skimmed milk powder have similar predisposing effects. This effect was significantly higher than the effect of vegetal-derived ingredients of MR. Therefore, it can be concluded that the predisposing effect of MR is due to the dairy ingredients. Further experiments are needed to identify the specific (combination of) component(s) of milk powder responsible for the observed predisposing effect on haemorrhagic enteritis in calves.

Internal Medicine

P03-003-104

Influence of enzootic bovine leukosis on immune response of naturally infected cattle

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Objectives: Enzootic Bovine Leukosis (EBL) is an infectious, chronic, neoplastic disease, which undermines the lymphopoietic organs and is associated with the development of persistent lymphocytosis (PL) and lymphosarcoma. Infected animals present a decrease of production, by its direct and indirect harmful effects. However, its effect on the function and quantity of lymphocyte subpopulations, and its role in the establishment of other opportunistic diseases, are unclear. We evaluated the immune response of Holstein cows infected with Bovine Leukosis Virus (BLV), after antigen challenge provided by vaccination against foot and mouth disease (FMD) virus.

Materials and Methods: Blood samples from ten seropositive cows without PL, from ten seropositive cows expressing PL, and from ten seronegative cows were collected before challenge and after challenge, weekly, for seven weeks. We evaluated the quantitative changes of different subpopulations of leukocytes; the function of B lymphocytes, through the quantification of different isotypes of immunoglobulins (Ig) serum concentration; the rate of lymphocyte proliferation; the rate of cell death by apoptosis or necrosis; and the serum concentrations of interleukin-10 (IL-10), IL-12, interferon- γ (IFN- γ), and tumor necrosis factor- α (TNF- α). The normality of distribution of the results was verified using the Anderson-Darling test, and their homoscedasticity, using the F test (for data with normal distribution) or the Levene test (for data without normal distribution). For the evaluation of differences between the average results, according respectively to the presence or absence of homoscedasticity, we used, for data with normal distribution, One-way ANOVA test, followed by the Tukey-Kramer test or the t test, and, for data without normal distribution, the Mann-Whitney test or the Kruskal-Wallis test. Results with $p \leq 0.05$ were considered statistically significant.

Results: No differences in IgG1, IgM, and IgA concentrations were found among sampling time and, every time, among groups. IgG2 increased after challenge (T0) in all cows ($p < 0.05$). Cows with PL, each time, since 17 days after T0, IgG2 had lower ($p < 0.01$) than cows of other groups, indicating that cows with PL present less intense and less enduring humoral response. An increased rate of lymphocyte proliferation ($p < 0.01$) 24 days after T0 was found in all cows. Since then, there was an increase in the percentage of $\gamma\delta$ -lymphocytes ($p < 0.05$) and a later decrease in serum IgG2 ($p < 0.05$). Cows with PL had higher $\gamma\delta$ -lymphocytes percentage ($p < 0.05$), possibly leading to the more intense and earlier

decrease in IgG2. PL was due to a lower rate of apoptosis, since the percentage of leukocytes undergoing apoptosis was lower ($p \leq 0.001$) in cows with PL than in other groups. Th1 cytokines (IL-12 and IFN- γ) were higher in non-PL infected cows ($p < 0.01$), whereas Th2 cytokines (IL-10 and TNF- α) were higher in infected cows with PL ($p < 0.01$). IL-10 ($p < 0.01$), TNF- α ($p = 0.005$), and IFN- γ ($p < 0.01$) increased three days after T0, and IL-12 increased ($p < 0.001$) ten days after T0. The increase in IL-10 lasts until 31 days after T0 and may account for the higher rate of $\gamma\delta$ -lymphocyte proliferation found after 31 days since T0. The majority of circulating B lymphocytes in cattle was B1 lymphocytes and, in BLV-infected cows, PL occurred due to an increase in the percentage of B1a lymphocytes ($p < 0.05$).

Conclusions: Results allow us to conclude that lymphocytotic BLV-infected animals have impaired immune response after an antigenic challenge, represented by the vaccination against FMD virus. IgG2 serum concentration after the challenge indicate that cows with PL present less intense and less enduring humoral response, probably led by their higher increase in the percentage of circulating $\gamma\delta$ -lymphocytes. Moreover, PL is due to a lower rate of apoptosis, rather than to a higher rate of lymphocyte proliferation, and occurs due to an increase in the percentage of circulating B1a lymphocytes.

Internal Medicine

P03-003-105

An abattoir study of ruminoreticular adhesions and retention rate of rumen magnets in cattle at slaughter

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Objectives: Although there are numerous reports of traumatic reticuloperitonitis (TRP, 'wire') in cattle, we are unaware of any UK studies of the prevalence of the condition, although a Danish abattoir study of 1491 dairy cows found that 16% had foreign bodies in their reticulum and of these 10% were associated with lesions. As rumen magnets cost only £2 per animal, if they are effective, routine dosing must be an extremely sensible precaution. The current study assessed the abattoir prevalence of TRP, and the retention rate of rumen magnets.

Materials and Methods: An initial postal survey of 56 dairy farms in the Gloucester area identified the number of dairy farms administered magnets to all cows as a routine. These farms were then invited to send cows to a local abattoir on 3 specific days, and they were examined at slaughter with other cattle submitted. At slaughter, the rumen, reticulum, omasum, abomasum, spleen and liver of 373 cows, 137 dairy and 236 beef, were visually assessed and palpated by an assessor, and organs with adhesions and/or abscesses were recorded and classified (acute/fibrinous, chronic/fibrous, or a mixture). A second assessor searched the rumen and reticulum for magnets. The age and breed was taken from abattoir and passport records.

Results: Only 30% of farms administered magnets to all cows. Of the 373 cows examined at the abattoir 91 (24.4%) showed lesions of peritonitis, 47 (34.3%) dairy and 44 (18.6%) beef cows. Many of the lesions were very mild, although frank abscesses were visible in 23 (6.2%) of the cows overall, 14 (10.2%) of the dairy animals and 9 (3.8%) beef. The significant association between breed of cow (dairy versus beef) and presence of reticuloperitonitis lesions ($P < 0.001$) confirms that dairy cows in are at increased risk of TRP. Magnets were detected in the rumen or reticulum of 29 (21%) of dairy cows and none in beef cows. When comparing the lesions in dairy cows with and without magnets, there was

no difference in the level of peritonitis, but there were no abscesses in cows with magnets but 14 (13%) of the 108 dairy cows without magnets had abscesses. Thirty-nine cows from four farms were examined where the farm stated that all cows had received a magnet prior to slaughter. Of these, only 24 (61.5%) contained a magnet at the abattoir.

Conclusions: Although dairy cows were worst affected, both dairy and beef animals had lesions suggestive of TRP. There was a significant association between magnet absence and presence of non-reticular peritoneal adhesions ($P < 0.05$) and all adhesions in the cows with magnets were reticular only and no abscesses were present. This suggests that magnets can inhibit foreign body migration from the reticulum, supports their use both therapeutically and prophylactically, and suggests that more widespread use would improve both the welfare and productivity of dairy and beef cattle.

Internal Medicine

P03-003-106

Physicochemical Approach To Acid-Base Disorders In Hospitalized Cattle

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Objectives: The objectives of this retrospective study were to determine the main acid-base disorders observed in hospitalized cattle using the physicochemical and the traditional Henderson-Hasselbalch (H-H) approaches and also to compare their diagnostic usefulness.

Materials and Methods: An eight-year retrospective study was conducted at the Large Animal Hospital, Faculty of Veterinary Medicine and Zootechny of the National University of Colombia that involved all the medical records of bovine patients admitted during that period. The inclusion criteria in the study were that the patient had to have complete results of arterial or venous blood gases, serum electrolytes (Na^+ , K^+ , Cl^-), and total plasma protein (TPP) from samples taken before any treatment was administered at the hospital. On each history, signalment, main complaint, working diagnosis, biochemical profile and hemogram were also collected. In all the cattle that met the inclusion criteria, venous or arterial blood was collected into a Na-heparin blood gas syringe and analyzed for pH, pCO_2 , calculated HCO_3^- and Base Excess (BE) using a blood gas analyzer, serum concentrations of Na^+ , K^+ , Cl^- were measured in venous blood using an ion-selective electrode analyzer and TPP were measured using a refractometer. Anion Gap (AG) was calculated as: $(\text{Na} + \text{K}) - (\text{Cl} + \text{HCO}_3^-)$; Strong ion difference (SID) and Strong ion gap (SIG) were calculated as: $\text{SID}_3 = (\text{Na} + \text{K}) - (\text{Cl})$, $\text{SIG} = \text{A}^- - \text{AG}$. A^- is net negative charge of plasma proteins; $\text{A}^- = 0.22x [\text{total protein, g/L}] / (1 + 10^{(\text{pKa} - \text{pH})})$.

Results: Only thirty-one patients of the 245 hospitalized bovine met the inclusion criteria in the 8-year period. Out of the 31 patients, 16 had arterial (A) blood gases measured and in 15 they were measured in venous (V) blood. The values observed were: $\text{A} \text{pH} = 7.37 \pm 0.15$; $\text{V} \text{pH} = 7.39 \pm 0.15$; $\text{A} \text{pCO}_2 = 39.82 \pm 15.19 \text{ mmHg}$; $\text{V} \text{pCO}_2 = 39.81 \pm 9.32 \text{ mmHg}$; $\text{A} \text{HCO}_3^- = 23.02 \pm 7.95 \text{ mmol/L}$; $\text{V} \text{HCO}_3^- = 26.2 \pm 10.55 \text{ mmol/L}$; $\text{A} \text{BE} = -2.25 \pm 9.61 \text{ mmol/L}$; $\text{V} \text{BE} = 1.33 \pm 12.69 \text{ mmol/L}$; $\text{Na} = 135.35 \pm 12.72 \text{ mmol/L}$; $\text{K} = 4.04.1 \pm 1.01 \text{ mmol/L}$; $\text{Cl} = 96.71 \pm 15.47 \text{ mmol/L}$; $\text{SID} = 37.49 \pm 10. \text{ mmol/L}$; and $\text{A}^- = 12.83 \pm 2. \text{ mmol/L}$; $\text{AG} = 18.01.7 \pm 8.88 \text{ mmol/L}$; $\text{SIG} = -5.17 \pm 8.24 \text{ mmol/L}$. Using the H-H approach acid base abnormalities were found in 83.3% of the patients compared to 93.5% using the physico-chemical approach. 61.3% had SID acidosis, 38.7%

had hyperproteinemic acidosis, 16.12% had SID alkalosis and 38.7% had hypoproteinemic alkalosis.

Conclusions: The physicochemical approach allowed detecting more acid base disturbances in hospitalized bovines than the H-H approach. These results indicate the need to determine accurately the acid base disorders in hospitalized cattle for their adequate treatment.

Internal Medicine

P03-003-107

Practic application of thoracoscopic lung biopsy in bovine respiratory disease

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Objectives: The thoracoscopy is an easy and safe diagnostic technique, already used in healthy bovines. This technique allows sample collection directly from diseased intrathoracic organs (biopsy). If the thoracoscopy can be safely used to take lung biopsies from BRD-diseased animals, the specific etiological cause of the particular lesions could be approached. This would be of huge interest for the bovine medicine and the bovine productive sector.

Hence, the aim of the study was to evaluate the quality of lung samples obtained through thoracoscopic biopsy and the influence of specific factors (sex, weight, and lung lobe) on the quality of such samples.

Materials and Methods: Lungs from 40 bovines (experiment 1) obtained at slaughterhouse were postmortem biopsied with the thoracoscopic equipment based on flexible endoscopes and lung samples quality and potential factors influence analyzed. In experiment 2 diagnostic information from in vivo samples (including thoracoscopic biopsies) coming from three BRD-diseased animals were compared with ultrasonography, classical exploration, and laboratory analyses of bronchial alveolar lavage, nasopharyngeal swab and blood analyses.

Results: A total of 91.25% (73/80) of valid samples was obtained. Samples taken from cranial lobes included significantly more often lung tissue (11.6%; $P = 0.0038$) than caudal lobes. Samples taken from lighter (<250Kg) animals tended to have more often valid samples, with more parenchyma (1803,7±1069,8 vs. 1255,3±886,7 microns/1000; $P = 0.038$) than heavy animals (≥250Kg). These differences could be caused by the different pleural characteristics observed in cranial vs. caudal lung lobes (166.2±45.5 vs. 194.1±77.5 microns of pleural width; $P = 0.112$), in lighter vs. heavier animals (162.6±60.5 vs. 186.6±64.4 microns of pleural width; $P = 0.212$), in female vs. male (556.4±445.6 vs. 652.2±534.5 microns/1000 pleural surface, $P = 0.14$) and in <14mo vs. ≥14 mo old (488.0±387.1 vs. 688.5±538.1 microns/1000 pleural surface; $P = 0.113$). The macroscopic estimation of the pulmonary tissue based on the observation of pinkish speckled appearance performed by the endoscopist resulted to foresee the existence of pulmonary tissue (Se=97.2%; Es=71.4X%; k value=0.687 and $P < 0.0001$), which has a great practical value as it allows deciding intraoperatively the repetition of the biopsy.

A total of 95.23% (20/21) of valid samples was obtained and a better diagnostic capacity was demonstrated for the thoracoscopy and thoracoscopic biopsy, when compared to ultrasonography, classical exploration, bronchial alveolar lavage, nasopharyngeal swab and blood analyses.

Conclusions: Complete and descriptive information about characteristics of the lesions and the causal agents were available, even in the case of *Mycoplasma bovis*, with a notably better diagnostic efficacy than with other analytical techniques applied. Based on these results, thoracocopy and thoracoscopic lung biopsy are demonstrated as safe, easy, useful and better diagnostic tools for BRD-diseased animals.

Internal Medicine

P03-003-109

Inducing abomasal lesions with phenylbutazone: a case report

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Objectives: Abomasal ulcers cause economic losses but little information about diagnosis and treatment is available, especially for adult ruminants. Omeprazole and ranitidine are the mucosal protectors commonly used in veterinary medicine, but there are no specific recommendations for polygastric animals. We studied sheep to evaluate the preventive action of ranitidine and omeprazole and identify markers for abomasal lesions caused by phenylbutazone administration.

Materials and Methods: Phenylbutazone was defined as the most ulcerogenic non-steroidal anti-inflammatory drug for goats treated twice a day for 12 days. Respecting the half-life of phenylbutazone in ovines of approximately 18 hours, daily intravenous administration of 4mg/kg of body weight were made in four healthy adult sheep fitted with abomasal cannulas. Endoscopic images obtained directly from the abomasum, blood samples and the physical parameters were collected daily from the first day of phenylbutazone administration up to a maximum of 14 days of this induction.

Results: On the 2nd day of phenylbutazone administration one sheep showed apathy, ruminal atony and anorexia; the serum fibrinogen was 6g/L, the abomasal pH was 2.52 and the acid secretion, 2.4 mL NaOH 0,1N. On the 3rd day, rounded lesions on the body portion of the abomasum appeared; the cardiac frequency was 128 beats per minute, the respiratory frequency was 44 breathes per minute and the animal showed two complete and one incomplete ruminal contraction during three minutes observation. The fibrinogen concentration remained 6g/L, the serum pepsinogen was 4.5UI, the abomasal pH was 2.88 and the acid secretion, 7.08 mL NaOH 0,1N. On the 4th day, this animal showed cardiac and respiratory frequencies a little higher than the normal, but the other parameters were adequate. The image of the abomasum showed a great improvement in the appearance of the mucosa. On the 5th day the endoscopic examination showed up again with rounded lesions in the body region and in the afternoon the animal presented severe discomfort, mainly noticed by tachypnea and the unusual position in which the animal was lying. Because of these signs on the next day the sheep did not receive the anti-inflammatory drug. The endoscopic images revealed significant diffuse lesions in the abomasum, in a quantity impossible to quantify. Ruminal movements were greatly decreased and weak and the animal was quiet. The abomasal pH was 3.05 and the acid secretion, 6.28 mL NaOH 0,1N. From the 2nd day without phenylbutazone administration the abomasum was apparently clean of lesions and ruminal movements were restored.

Conclusions: In the observed case only removing the stimulus of the injury was enough for the spontaneous recovery of abomasal mucosa in one day. The other animals used didn't show macroscopic abomasal

damage until the 9th day so the administration of phenylbutazone started to be done twice a day from the 10th day. No macroscopic damage in the abomasum, appetite decrease or clinical parameters alterations was observed until the 14th day of phenylbutazone administration. The difficulty to induce abomasal ulcer shows that healthy and well-fed ovine tolerate phenylbutazone in the dose and during the time used better than expected.

Internal Medicine

P03-003-110

BOVINE TRYPANOSOMOSIS: HAEMATOLOGY, SERUM GLUTAMIC OXALO-ACETIC TRANSAMINASE (SGOT) AND CREATININE (CRT) LEVELS IN THREE BREEDS OF NIGERIAN CATTLE

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Objectives: Trypanosomosis is endemic and it constitutes a hindrance to livestock productivity in Africa. Although anaemia is a prominent feature of natural bovine trypanosomosis, its effects on serum glutamic oxalo-acetic transaminase (SGOT) and creatinine (CRT) levels in Nigerian cattle have not been researched. This study was therefore carried out to determine the effects of natural bovine trypanosomosis on the haematology, serum glutamic oxalo-acetic transaminase (SGOT) and creatinine levels in some breeds of Nigerian cattle.

Materials and Methods: A total of 243 cattle comprising 88 apparently healthy and 155 trypanosome-infected cattle (without other blood and helminth parasites) of both sexes among Sokoto Gudali, Red Bororo and White Fulani breeds, were used for the study. The animals were sampled from three different farms from Ibadan over a period of nine months. Dentition technique was used to estimate ages of the cattle sampled. Modified McMaster technique was used to ascertain levels of helminthosis. Buffy coat and Giemsa staining techniques were used to make diagnosis and determine morphology of the trypanosomes. The Packed Cell Volume (PCV), Haemoglobin Concentration (Hb) and Red Blood Cell Counts (RBC) were determined using poch-100iV Diff (Sysmex Animal health), while the serum SGOT levels were determined using Randox[®] kits (Randox Laboratories, UK) and CRT levels were determined using Serum creatinine kit (Fortress Diagnostics, Ltd UK). All data obtained was subjected to descriptive statistics and SPSS statistical package version 9.0 at a significance level of 5%.

Results: Mixed infections with *Trypanosoma congolense* and *Trypanosoma brucei* were recorded. Across all the breeds of cattle, there was anaemia with significantly ($P < 0.05$) decreased values of PCV, Hb and RBC. A significant ($P < 0.05$) increase in the value of serum enzyme concentration of SGOT and CRT was obtained in the naturally infected cattle across the breeds. Cattle above two years of age were found susceptible to natural trypanosomosis across the three breeds. There was no sex disposition in both the haematological profile and serum SGOT and CRT values

Conclusions: The deleterious effects of trypanosomes on the haematology of cattle is reaffirmed while it is suggested that leakage of enzymes from their intracellular stores due to trypanosome-induced cell membrane disruption, increased production in the liver and/or decreased excretion through the glomerular filters might have resulted from hepatotoxicity and nephrotoxicity due to parasite derived toxic factors. Cattle farmers in Nigeria have been advised appropriately.

Internal Medicine

P03-003-111

Injection Site Protocols: Bacterial Loads on Skin of Unclipped Gluteal Sites Following Treatment with 70% Isopropyl Alcohol-Soaked Swabs in Dairy Cows

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Objectives: Intra-muscular injection in cattle is a common procedure in cattle practice. There is a wide variety of bacterial flora present on cattle skin which may lead to abscess formation and carcass damage following contamination of the needle at the time of injection. There is no evidenced-based protocol for the treatment of skin before an intra-muscular or sub-cutaneous injection is administered in cattle. The aim of this study was to compare the bacterial load of unclipped gluteal skin in dairy cows following either no treatment or treatment with a standard 70% isopropyl alcohol-based skin treatment protocol.

Materials and Methods: Twenty Holstein-Friesian dairy cows from a commercial dairy herd in Cambridgeshire, England, were used in this randomised, blinded, controlled study. On each of the experimental cows an area of unclipped gluteal skin on one side of the pelvis was treated with swabs soaked in 70% isopropyl alcohol-based using a standard protocol. A contra-lateral area of skin was left untreated as a control. All the experimental skin sites were sampled using a swab followed by bacterial culture and quantitative analysis of bacterial load.

Results: There was a statistically significant decrease in the bacterial colony forming units per mL for the isopropyl-alcohol treatment group when compared to the control group ($p \leq 0.01$, Mann-Whitney U-test). There was a 58% reduction in the median bacterial load of the treated sites when compared to the bacterial load of the untreated sites. There was no statistically significant difference between either the right and left treatment sites ($p > 0.05$, Mann-Whitney U-test) or the right and left control sites ($p > 0.05$, Mann-Whitney U-test).

Conclusions: This study has demonstrated that a treatment protocol with swabs soaked in 70% isopropyl alcohol can substantially reduce the skin bacterial load but not eliminate all bacteria. This study supports the use of skin swabbing prior to injection in-order to reduce the risk of needle contamination.

Internal Medicine

P03-003-112

Agreement among sampling methods used to identify viral and bacterial pathogens in dairy calves with naturally-occurring respiratory disease

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Objectives: Bovine respiratory disease (BRD) is common in dairy calves, and antemortem sampling to identify respiratory pathogens is often undertaken. Sampling methods used include the nasal swab (NS), deep guarded nasopharyngeal swab (NPS), transtracheal wash (TTW), and bronchoalveolar lavage (BAL). However, the agreement among these tests has not been well characterized. The objective of this study was to compare the agreement of results obtained by NS, NPS, or BAL with those obtained by TTW for identification of bacterial and viral respiratory pathogens in pre-weaned dairy calves with naturally occurring BRD.

Materials and Methods: Calves at a single calf rearing facility with naturally occurring respiratory disease in the first 90 days of life were enrolled if they had a score of 5 or greater on the University of Wisconsin Calf Respiratory Scoring Chart, a fever of 103°F (39.4° C) or higher, and at least 2 cm of pulmonary consolidation identified by transthoracic ultrasound. Calves that had ever been treated for respiratory disease, or that had received intranasal modified live viral respiratory vaccine in the previous 30 days, were excluded. A NS, NPS, TTW, and BAL were collected sequentially from each calf using standard methods. Bacterial pathogens were identified by aerobic bacterial and mycoplasma culture, and viral pathogens were identified by real time RT-PCR. Mycoplasma sp. isolated were subjected to PCR to specifically identify *M. bovis*. Agreement between the results of TTW and the NS, NPS, or BAL was determined by calculation of the kappa statistic. Values of kappa were categorized according to these levels of agreement: < 0.21 = poor; $0.21 - 0.40$ = fair; $0.41 - 0.6$ = moderate; $0.61 - 0.80$ = good, and $0.81 - 1.00$ = very good.

Results: One hundred calves were enrolled. No samples were positive for bovine herpesvirus-1 (BHV-1), bovine viral diarrhoea virus (BVDV), or *Histophilus somni*. Nearly all samples were positive for *Mycoplasma* sp., leading to low values for kappa for *Mycoplasma* sp. Relative to the TTW, all sampling methods showed very good agreement for identification of *Pasteurella multocida*, *Mannheimia haemolytica*, or *Mycoplasma bovis*. In contrast, for identification of bovine respiratory syncytial virus (BRSV) and relative to TTW, agreement was moderate for NS, good for NPS, and very good for BAL. For identification of bovine coronavirus (BCV) and relative to TTW, agreement was moderate for the NS and NPS, and good for BAL.

Conclusions: Agreement between TTW and other sampling methods differed for different pathogens. For bacterial pathogens, agreement was very good for NS, NPS, and BAL. For BRSV and BCV, results of BAL agreed best with the TTW.

Internal Medicine

P03-003-113

An atypical case of cutaneous and respiratory actinobacillosis in a French beef cattle herd

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Objectives: Actinobacillosis is a worldwide infectious disease due to *Actinobacillus lignieresii*, generally considered as a sporadic condition typically involving the tongue ("wooden tongue") and draining lymph nodes in cattle. We present here another and very different clinical pattern.

Materials and Methods: Two "Rouge-des-Prés" beef cattle from a single herd were referred with a common history of chronic evolution of a severe dermatosis affecting, and limited to, hind legs. The general examination revealed cachexia, signs compatible with pneumonia, regional lymphadenitis and burst abscess in the udder of one cow. Cutaneous signs were characterized by multiple single to coalescent cutaneous and subcutaneous nodules (0.5 to 10 cm in diameter), some of them ulcerated with crusts. It was combined with massive swelling of the affected limbs and numerous releasing pus fistulae. The differential diagnosis included first a cutaneous infection due to an Actinomycetes organism or possibly another group of bacteria secondary to a primary parasitic (Besnoitia or leg mange), deep fungal or viral (lumpy skin disease) aetiology.

Results: Direct smears using rapid stains were inconclusive and no parasite was observed in multiple skin scrapings. Fungal cultures were negative. Two series of biopsies were performed and histopathological examination revealed chronic, diffuse, severe pyogranulomatous dermatitis, associated with Splendore-Hoeppli phenomenon Gram-negative bacteria and minute bacterial grains compatible with mycetoma/pseudomycetoma. Special stain (Ziehl-Neelsen) was negative for *Mycobacterium* spp.. Poxviruses PCR was negative. Cultures from skin, lymph nodes and lungs (both cows were euthanized for welfare reasons) identified a Pasteurellaceae organism, confirmed as *Actinobacillus lignieresii* by partial sequencing of the *rpoB* gene with 99.4% DNA similarity to the type strain of the species. Two strains sequenced from the herd with origins in pus from a cutaneous nodule and a mammary lymph node, respective showed 99.4% DNA similarity indicating that the infection in the herd was not related to a single clone.

Conclusions: One other similar case was observed in the same herd suggesting a pseudocontagious, likely environmentally induced, condition, thus illustrating that widely distributed organisms like *Actinobacillus* may be associated to non-classical clinical presentation.

Internal Medicine

P03-003-114

A Likely Case Of An Outbreak Of Theileriosis In Simmental And Holstein Heifers: First Report In Colombia

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Objectives: The objective of this study is to present the first report of a likely outbreak of bovine theileriosis which is an exotic disease in Colombia and to describe the clinical features observed as well as the treatment implemented.

Materials and Methods: Two 22 month-old pregnant Holstein and Simmental heifers that weighed 380 Kg were referred to the Large Animal Clinic at the National University of Colombia with history of depression, pigmenturia and dark feces. They were treated with oxytetracycline, diminazene, vitamin B and flunixin meglumine. The heifers came from a farm with animals of different breeds on pasture and supplemented with

concentrate and corn silage. 20 days before admission, a Simmental adult cow and a heifer with similar clinical signs were treated with oxytetracycline/imidocarb and died within 2 days despite treatment. Hematological evaluation requested by the referring veterinarian of other Zebu cattle showed PCV values between 20 - 22% and the blood smears revealed the presence of *Babesia* spp in 30% of the heifers and 50% of the female calves, fecal exams revealed low parasite burden and no diarrhea was observed.

On clinical examination, the heifers were in recumbency, unable to get up, had low body condition score (2/5), sunken eye bulbs (15 mm), severe dehydration (10-12%), pale and icteric mucous membranes, weak pulse, increased capillary refill time (5"), tachycardia (86 and 160 BPM), tachypnea (16 and 52 breath per minute), hypothermia (36.7°C), and in one animal, ruminal atony, pigmenturia, increased paralumbar lymph nodes were observed. Rectal palpation of both patients indicated lack of feces and collapsed rumen. Ticks were observed also on both patients. Venous blood was collected for CBC, serum biochemistry and blood gases; urine was sent for urinalysis and an ECG was done on one heifer.

Results: CBC showed a severe anemia in both animals (PCV: 9 and 10%), hypoproteinemia and lymphopenia. In both cases, microscopic structures compatible with *Theileria* spp. were observed in high numbers in lymphocytes and red cells and neither *Babesia* spp. nor *Anaplasma marginale* were observed. Serum chemistry tests showed increased urea, AST, Cl⁻ and lactate levels in both heifers as well as a decrease in K⁺, ionized Ca⁺⁺, Glucose and tCO₂; only one heifer showed increased GGT and Na⁺. Blood gases and electrolyte evaluation showed hyperchloremic and lactic acidosis. Urinalysis revealed aciduria, hemoglobinuria and proteinuria. ECG on the Holstein heifer indicated a ventricular tachycardia.

Both cases were treated with 7.5% hypertonic saline solution, isotonic saline to treat dehydration and 5% NaHCO₃ solution to correct acidosis. To treat the hemolytic anemia blood transfusions were performed. Calcium borogluconate was administered to correct the hypocalcemia and flunixin meglumine as antiinflammatory medication. To treat the theileriosis a combination of oxytetracycline and diminazene was administered several times given that no buparvaquone is available in Colombia. The ventricular tachycardia was corrected using IV lidocaine and vitamin B complex was used as hematinic. To treat the *Boophilus* spp. tick infestation ivermectin was given and ruminal transfaunation was performed to stimulate appetite. After these therapies, hematological, serum and acid base values returned to physiological ranges. The two heifers were discharged from the clinic 29 days after admission.

Conclusions: In conclusion, it is a very likely outbreak of Theileriosis in Holstein and Simmental heifers and the first report of this exotic disease in Colombia and given the clinical features, it may have been caused by *Theileria orientalis*.

Internal Medicine

P03-003-115

Maternal, Fetal And Neonatal Heart Rate And Heart Rate Variability Assessment In Holstein Cattle

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Objectives: Fetal heart rate monitoring not only enables verification of fetal health and viability, but also provides information about the stage of development of the autonomic nervous system. Thus, the aim of the work was to determine values in heart rate and heart rate variability in Holstein cows, her fetus and neonates.

Materials and Methods: The study was design to be descriptive of continuous fetomaternal and neonatal electrocardiogram recordings during perinatal and neonatal period. In the study, 23 healthy pregnant Holstein cows and 18 neonates were used. The electrocardiograms were registered during the period from May to November 2014. This project had the approval of the Ethics Committee on Animal Use - CEUA and signing the informed consent by the owner of the farm. Heart rate (HR) and heart rate variability (HRV) were assessed by fetomaternal electrocardiography (ECG). The electrocardiographic examinations followed a pattern of repetitions over time at pre-defined times. Fetomaternal recordings were conducted in six pre partum moments; 35, 28, 21, 14, 7 and 1 day, and in neonates six moments; 35, 28, 21, 14, 7 and 1 day after calving. Heart rate, time domain variables; the mean beat-to-beat interval (RR interval), its standard deviation (SDNN), and the root mean square of successive RR differences (RMSSD) and frequency domain variables; low frequency (LF), high frequency (HF) and ratio LF/HF of heart rate variability were analyzed. Changes over time were analyzed using a general linear model for repeated measures. A p value < 0.05 was considered significant. All data given are means \pm SEM.

Results: No significant changes in maternal and fetal RR interval and HR were found. In fetus, SDNN decreased significantly from 38.0 ± 2.6 ms to 28.9 ± 2.4 ms ($p < 0.05$), while RMSSD did not reach statistical significance. Fetal heart rate and interval RR differs statistically from one day before delivery (163 ± 7.5 bpm; 381 ± 24.2 ms) and the day after calving (131 ± 5 bpm; 472 ± 16.2 ms). Variables in time (SDNN and RMSSD) and frequency (LF and HF) domain presented a significantly difference ($p < 0.05$) from fetal to neonatal stage.

Conclusions: Fetomaternal ECG is a reliable technique to detect cardiac signals in bovine fetuses in the last 35 days of gestation. In the last week of gestation, reductions in the fetus values of SDNN and RMSSD reflect a shift towards more sympathetic dominance. After calving, the increased in high frequency and decreased in low frequency indicate activation of the vagal nerve on the heart and respiration modulation.

Internal Medicine

P03-003-116

Potassium Concentrations In Plasma, In Erythrocytes And Muscle Tissue In Cows With Decreased Feed Intake And Gastrointestinal Ileus

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Objectives: This method however may not be able to provide information about the intracellular potassium pool. The aims of the study were to evaluate the potassium metabolism by comparing the extracellular and intracellular potassium concentration in cows with reduced feed intake and gastrointestinal ileus and to determine the association between these two. Further the study aimed to investigate if cows with reduced feed intake suffer from potassium depletion or simply shift potassium between intra and extracellular space.

Materials and Methods: Twenty cows between day 1 and 3 days post partum and 20 cows with gastrointestinal ileus were included in the study. The cattle were Simmental and Holstein-Friesian cows. Venous blood was taken and plasma potassium concentration was measured using an ion sensitive electrode. A muscle sample of the gluteal muscle was taken by biopsy using a Bergstroem needle. Intracellular potassium concentration was measured in erythrocytes and muscle cells applying inductively coupled plasma optical emission spectroscopy.

Results: The diet of cattle contains large amounts of potassium therefore in cattle with undisturbed feed intake potassium is abundantly available. However, a decreased feed intake may result in hypokalemia. The routine method to evaluate potassium metabolism is the measurement of the extracellular potassium concentration in plasma or serum. Postpartal cows did not show hypokalemia, cows with gastrointestinal ileus were hypokalemic (2.9 mmol/l \pm 0.78). Some of the hypokalemic cows showed muscle tremor, but no other signs of paralysis were observed. There was no difference of potassium concentrations in erythrocytes and muscle tissue between hypokalemic and normokalemic cows. Intracellular potassium in erythrocytes varied very widely; cows post partum: 3497 to 10735 mg/kg, cows with ileus: 4139 to 21678 mg/kg. Potassium in muscle tissue did not differ between both groups. No association between extracellular and intracellular potassium concentrations could be found.

Conclusions: The results of the study indicate that measurement of plasma potassium concentrations is not suitable to evaluate potassium metabolism. In addition to extracellular potassium measurement muscle tissue potassium is needed to provide information about the intracellular potassium pool. Since other factors like blood groups influence potassium concentrations in erythrocytes these cells although easily obtainable are not suitable to measure intracellular potassium concentrations. Acute decrease of feed intake as it is the case in cows with ileus does not result in immediate potassium depletion but in a hypokalemia.

Internal Medicine

P03-003-117

Evaluation of abomasal, urinary and fecal pH, blood gas and chlorine ions in healthy sheep treated with oral omeprazole, preliminary data.

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Objectives: Abomasal ulcers often are unnoticed by farmer and veterinarians due the mild clinical manifestations and due the little available diagnostic resources. When the diagnosed is already established or there is clinical suspicion, the treatment is also restricted. Although studies are limited and there is no indication for ruminants, oral omeprazole often is used in veterinary hospitals to treat and / or prevent abomasal ulcers in these species. The objective of this study was to evaluate the effect of omeprazole in healthy sheep by physical examination; abomasal, urinary and fecal pH; blood gas and chlorine ions.

Materials and Methods: Five adult healthy sheep, males, castrated, average weight of 45 kg, with abomasal cannula were used. The animals were allocated in individual stalls and were fed a diet with hay and commercial concentrate in the proportion 80:20. Water was available ad libitum for intake and feed offered in 3% of body weight on a dry basis.

The animals were randomized in two groups. One group received oral omeprazole in the form of paste, at a dose of 4mg / kg every 24 hours for seven days and the other was untreated. The experimental design was cross-over type, with a washout period of seven days.

Physical exams and collections were performed in the morning on seventh day when sheep were fasted for 12 hours. Samples of faeces, urine and abomasal content were immediately subjected to the determination of pH. Blood samples were collected by jugular vein puncture for immediate determination of chloride, blood pH, concentration of bicarbonate (HCO₃) and base excess (BE).

Results: The use of oral omeprazole for seven days in sheep did not influence the abomasal pH (P = 0.357), urinary pH (P = 0.2963) and fecal pH (P = 0.678). There was also no significant effect on blood gas parameters. There was no effect on blood pH (P = 0.416), HCO₃ content (P = 0.8345) and base excess (P = 0.9168). Although also not statistically significant differences between sheep treated with omeprazole and control sheep, in physical examination there was a trend (P = 0.110) lower respiratory rate to those treated with omeprazole. There were no differences between groups for heart rate (P = 0.4647).

Conclusions: In healthy sheep the omeprazole administration at a dose of 4 mg / kg every 24 hours for seven days was not effective in increasing the pH abomasal. This drug not change the other parameters analyzed at the moment.

Internal Medicine

P03-003-118

Hematologic differences between calves and lactating dairy cows in Poland

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Objectives: Change in hematologic reference range in association with age have been described in cows. The purpose of this study was to determine whether CBC counts differ between calves and lactating dairy cows.

Materials and Methods: In this retrospective study, twenty clinically healthy calves were sampled for CBC. The calves included 14 males and 6 females. They were born between September and October, 2015. The blood were collected from the jugular vein into tubes containing K3EDTA (Venoject, Terumo, Leuven, Belgium). CBC data and total solids were compared between calves and lactating dairy cows (Mindray BC 2800 vet). Reference intervals were determined for 20 lactating dairy cows of the same breed.

Results: Twenty calves and twenty lactating dairy cows fitted the criteria for inclusion in the study. There was no evidence of a difference in sex frequency between calves or clinics. Calves had lower mean corpuscular volume (MCV), packed cell volume (PCV), red blood cells (RBC) count. There were marked differences in the distribution of hematocrit (HCT) and hemoglobin concentration (Hg) concentration between clinics (P<0,05). There were statistically significant but clinically unimportant differences in differential white blood cells (WBC) counts.

Conclusions: Compared with lactating dairy cows, calves have lower PCV, MCV, HCT, RBC count and HGB concentration. It is important

to be aware of such veterinarians when interpreting CBCs in calves to avoid misdiagnosis and unnecessary further diagnostic or treatment.

Internal Medicine

P03-003-119

Spinal Lymphosarcoma in a Dairy Heifer: A case report

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Objectives: The clinical and histopathological findings in a one-year-old Kiwi cross dairy heifer with compression of the spinal cord resulting from infiltration of a lymphosarcoma from the thoracic lymph nodes through the spinal nerve roots are described.

Materials and Methods: The heifer presented with paraparesis and apparent loss of body condition after being down on pasture for an unknown amount of time. On physical examination, reduced pain sensation in the pelvic limbs was evident, however a crawling forward motion could still be carried out. No hip, spine or limb asymmetry could be found, and all palpable superficial lymph nodes were within normal limits. Clinical biochemistry revealed mildly increased creatine kinase activity while electrolyte concentrations were within reference ranges. Despite symptomatic treatment with oral fluids and anti-inflammatories the heifer remained recumbent for four days, and was subsequently euthanized due to poor prognosis.

Results: On post-mortem examination, a large mass surrounded the vertebral bodies of the thoracic vertebrae (T) 10 to 13. The mass infiltrated the spinal canal via the spinal nerve roots, resulting in compression of the spinal cord. No bone lysis was present. A firm mass (5 cm x 5 cm x 10 cm) was also present on the right side of the rib cage. Numerous thoracic lymph nodes along the vertebral column were severely enlarged while the abdominal lymph nodes were mildly enlarged. Histopathological findings included severe wallerian degeneration with swollen axons in the spinal cord, and the thoracic and vertebral masses consisted of a monomorphic population of large lymphoblasts, consistent with lymphosarcoma.

Conclusions: Lymphosarcoma is an uncommon tumour of cattle, but a peak in incidence does occur in yearlings. A variety of clinical and paraclinical tests are often needed to establish a diagnosis of bovine leucosis, however lymphosarcoma should be included in the list of differential diagnoses for young cattle that present with paraparesis.

Internal Medicine

P03-003-120

Idiopathic pseudo pericarditis in Kangeyam bullocks – a clinical study

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Objectives: The term pseudo pericarditis has been used to describe similar symptoms of traumatic pericarditis caused by tumours, abscesses, echinococcus cysts, swollen lymph nodes resulting from tuberculosis and leukosis, one-sided pleuritis and diaphragmatic hernia. Because of the anatomic compactness of thorax and abdomen the pericarditis is rarely reported in *Bos Indicus* bullocks. This may be the first report in pseudo pericarditis in Kangeyam bullocks in India. This clinical report describes the clinical signs, diagnosis, treatment and clinical outcome in two cases.

Materials and Methods: This research was carried out at Teaching Veterinary Clinical Complex (TVCC), Veterinary College and Research Institute, Orathanadu – 614 625, India during October 2014 to December 2014. Two work bullocks bearing institutional case numbers 6636/14 & 6706/14 were reported with history of brisket and dewlap edema, anoxia, cessation of rumination, debility and admitted in large animal outpatient unit of TVCC. On clinical examination the animal had high temperature, engorgement of jugular vein, enlarged prescapular lymphnodes and ruminal atony. Survey radiography (Siemens, 320mA, high frequency machine with computed radiographic system) was taken to rule out the possibility of traumatic pericarditis. The complete blood count was carried out in automated hematology analyzer (Vet scan HM5, Abaxis) on every fourth day. Serum biochemistry was carried out in semi automated analyzer. Abdominocentesis was carried out in one animal (6636/14) and not in another (6706/14) because of owner's non-compliance. Both the animals were treated with Oxytetracycline antibiotic @ 20 mg/kg along with diuretics like frusemide @ 5 mg/kg, polyionic fluids like multiple electrolyte solution, ringer's lactate, 7.5% sodium bicarbonate, ruminatorics and B-vitamins. In both the animals rumen gut transplantation was attempted. Acetic acid-chalk paste was applied topically. Owners were advised to report any untoward reactions in animals.

Results: It was observed that *Bos Indicus* working Kangeyam bullocks were the most commonly affected by this disease than cross bred *Bos taurus* cattle. Similar observations were noted (personal communication) by veterinarians in the field. The clinical signs are anorexia from day one onwards, ruminal atony and cessation of rumination, dehydration, dull and depressed, fever, brisket edema which extends up to inter-mandibular region, jugular vein enlargement and enlarged prescapular lymphnode. The degree of dehydration was severe (7-9%). On cardiac auscultation the heart sounds were normal. Survey radiography revealed normal cardiac silhouette. There was a marked leukopenia and lymphopenia without neutrophilia on day one in both the animals. Shortly this turned to leukocytosis and lymphocytosis. There was a marked increase in globulin fraction with altered A:G ratio. Peritoneal fluid analysis ruled out the absence of traumatic peritonitis. Appetite was improved after 15 days of therapy (6636/14) along with rumination and after 10 days in another animal (6706/14). Complete recovery was noticed 20 and 15 days after therapy in 6636/14 & 6706/14 animals respectively

Conclusions: This clinical study concludes severity of pseudo pericarditis due to idiopathic origin possibly of increase in preload. The major clinical signs were brisket and dewlap edema, jugular enlargement, fever, anorexia, cessation of rumination, ruminal atony and inanition. Loop diuretic such as frusemide which decrease preload along with broad spectrum antibiotics, adequate fluid and electrolytes, sodium bicarbonate, gut transplantation, will help in clinical recovery of pseudo pericarditis animals.

Internal Medicine

P03-003-121

Efficacy of intravenous hypertonic saline solution in the treatment of experimentally induced neonatal diarrhea in buffalo calves

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Objectives: This study was conducted to determine the safety and efficacy of hypertonic saline solution (HSS) in the amelioration of hemodynamic parameters and other laboratory variables especially metabolic acidosis in buffalo calves with experimentally induced neonatal diarrhoea with *Escherichia coli*.

Materials and Methods: Twelve 5-8 days old healthy male buffalo calves (30–40 kg body weight) were instrumented under halothane anesthesia to permit hemodynamic parameters and collection of blood samples to observe different variables. Neonatal diarrhea was induced through oral administration of 2 ml broth culture of having enteropathogenic *Escherichia coli* count of 10¹⁰ CFU dissolved in 250 ml of normal saline solution. Calves were then randomly assigned to receive either isotonic saline solution (ISS; group A) @ 32 ml/kg BW or HSS (group B) @ 4 ml/kg BW. Hemodynamic parameters and other laboratory variables were monitored for 72 hours.

Results: Administration of hypertonic saline solution (group B) immediately corrected metabolic acidosis (base excess [BE] > -15 mM), restored mean arterial pressure and expanded the plasma volume more rapidly in calves of group B and showed significant difference (P < 0.05) over group A. Hypertonic saline treated calves had higher cardiac index after 1 hour of administration as compared to ISS treated calves and showed significant (P < 0.05). Administration of HSS also improved blood gases more rapidly and efficiently in calves of group B.

Conclusions: On the basis of findings of this study, it was concluded that hypertonic saline solution can be safely administered to the buffalo calves with neonatal diarrhea. It offset deleterious hemodynamic effects and provides a practical and economical method to resuscitate dehydrated calves with neonatal diarrhea associated with *E. coli*.

Internal Medicine

P03-003-122

Diagnosis of acute tubular necrosis secondary to *Amaranthus retroflexus* poisoning in cattle using urinalysis, fractional excretion and electrophoresis

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Objectives: Acute decline in kidney functions, also called Acute Kidney Injury (AKI), results in retention of nitrogenous (urea and creatinine) and non-nitrogenous waste products. The most common causes of AKI in bovine practice are prerenal (hypovolemia), and Acute Tubular Necrosis

(ATN) due to the damaging effect of toxins to renal tubular cells. In contrast to the companion animal species, few data are available in bovine on the most interesting biomarkers for characterization of AKI. The present report describes implementation of urinary and plasma biomarkers to achieve diagnosis of ATN caused by *Amaranthus retroflexus* poisoning.

Materials and Methods: Four sick adult cows (Blonde d'Aquitaine breed) were referred to the National Veterinary School of Toulouse because a history in the herd of unexplained sudden death in pasture. Clinical examination was performed and urine was obtained by bladder catheterization from each cow. Standard analysis was performed on urine and plasma (Li-heparinate, Vitros350, Ortho Clinical Diagnosis, England) using a chemical analysis with urinary strip (URIVET-100, Kitvia, France) completed by a Heller's test (nitric acid) to detect proteinuria, by Urine Specific Gravity (USG) estimation via refractometry and by unstained sediment examination. Additionally, Urine Protein / Creatinine ratio (UPC), fractional excretion of sodium ($FeNa = [(U Na * P Creat) / (P Na * U Creat)] * 100$), fractional excretion of urea ($FeUrea = [(U Urea * P Creat) / (P Urea * U Creat)] * 100$) were calculated using biochemical urine results (Kbio2, Kitvia, France). Urine protein electrophoresis on sodium dodecyl sulfate-agarose gel (SDS-AGE) was simultaneously performed.

Results: At first examination, clinical signs were anorexia, paresis, bradycardia, dark diarrhea and an expired breath smelling ammoniac. Plasma analyses revealed a severe azotemia (creatinine between 1692 and 2593 $\mu\text{mol/L}$), with hyperphosphatemia (between 2.25 and 5.21 mmol/L) and hypocalcemia (between 0.95 and 2.24 mmol/L), which, in combination with the clinical signs, suggested an AKI. To distinguish prerenal impairment from kidney injury, urine analyses were further performed. Urinary strips were consistently positive for protein, glucose and peroxidase activity but Heller's test was positive in only two of the four cases. Urine pH was in the normal ranges, while the USG was low, near isosthenuric. Free Renal Tubular Epithelial Cells (RTEC) and Renal Tubular Epithelial Cells casts or granular casts were detected on unstained sediment of urine. Urinary biochemistry revealed an increased sodium concentration (between 48 and 97 mmol/L), an increased UPC (between 1.30 and 2.70, canine reference value < 0.5), an increased FeNa (between 8.7 and 55.1%, bovine reference value $< 1\%$) and an increased FeUrea (between 83 and 120%, human reference value $< 35\%$). Urinary protein electrophoresis revealed low molecular weight proteins consistent with tubular damage. All these findings were highly suggestive of an ATN. Three of the four cows died and were necropsied. Microscopic lesions of kidney confirmed the diagnosis of ATN. A toxicological analysis of the rumen contents of the dead cows, combined with a pasture inspection, demonstrated that the ATN was associated to *A. retroflexus* ingestion.

Conclusions: AKI secondary to an acute tubular necrosis associated with *Amaranthus retroflexus* ingestion was confirmed by histopathological and toxicological results. The reported cases illustrate the value of the combination of urinalysis, fractional excretion of sodium, urine protein / creatinine ratio and urine electrophoresis for the diagnosis of ATN. Future research should focus on the establishment of specific thresholds for urinary biomarkers in cattle medicine.

Internal Medicine

P03-003-123

Polyarteritis nodosa in a three year old Belgian Blue cow

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Objectives: Polyarteritis nodosa, also known as Kussmaul disease, is a systemic vasculitis of small- or medium-sized muscular arteries, typically involving renal and visceral vessels but sparing the pulmonary circulation. The objective of the present study was to describe a case of polyarteritis nodosa in an adult cow.

Materials and Methods: A 3-year old female Belgian Blue cow was presented with the complaint of weight loss, fever and diarrhea since 4 weeks. The cow gave birth to a healthy calf via cesarian 3 months prior. The cow had been treated with antibiotics, NSAID's, and imidocarb at home without improvement. Clinical examination, blood examination, ultrasound, urine analysis, autopsy, bacteriology and histology of the heart, liver, intestine, kidney, brain, spleen, lung, pancreas and lymph nodes were used in the diagnostic work-up of this case.

Results: Clinical examination revealed cachexia, mild fever (39,2°C), severe diarrhea, congested mucosae and dehydration. On biochemical analysis total protein and kidney-values were normal, but gamma-glutamyltransferase (GGT) was severely elevated ($> 952\text{U/L}$, reference value 0-80U/L). Aspartate aminotransferase (AST), alkaline phosphatase, lactate dehydrogenase (LDH), creatine kinase (CK), and total bilirubin were mildly elevated. Blood gas analysis showed a mild metabolic alkalosis and the ionogram revealed hypokaliemia. Hematology was normal. Serology for *Fasciola hepatica* and Johne's disease were negative, as was antigen ELISA for bovine viral diarrhea (BVD). Ultrasound of the thorax revealed a more hyper echogenic aspect of the liver. Urine analysis showed no abnormalities. The cow was euthanized due to poor general condition. The main conclusions from necropsy were diffuse catarrhal enteritis, dehydration and cachexia. The liver showed mild rounded edges, but no other gross abnormalities. The heart had several subendocardial white necrosis spots. Bacterial swabs from the jejunum and mesenteric lymph nodes were polybacterial. Histology of the liver showed a mild fibrosis around the smaller bile ducts, as can be seen in fluke-infestation. The lamina propria in the intestine was infiltrated with mainly eosinophils and some lymphocytes and plasma cells, indicating an eosinophilic enteritis. The arterial wall from several big arteries in the heart, liver and kidney were infiltrated by mainly lymphocytes and plasma cells, and some neutrophils, indicating polyarteritis nodosa.

Conclusions: Polyarteritis nodosa arises when pathogenic agent damage the arterial wall, which leads to the activation of the Hageman factor. This will cause vasculitis, with chemotaxis of neutrophils, increased permeability, fibrosis, thrombosis and necrosis. The only confirmed cause of polyarteritis nodosa is hepatitis B in humans. In cattle, bovine malignant catarrhal fever (BMCF), BVD and sarcosporidiosis have been suggested as potential causes for polyarteritis nodosa. However, there were no signs of these diseases in this animal. The chronic presentation of the polyarteritis nodosa impedes the possibilities of identifying the primary etiology.

Internal Medicine

P03-003-124

Simultaneous outbreak associated with Bovine herpesvirus-5 and *Histophilus somni* in feedlot cattle from Southern Brazil

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Objectives: Bovine herpesvirus-5 (BoHV-5) is endemic in cattle herds from Brazil and is one of the principal causes of neurological disease. Histophilosis is a disease complex that is caused by the gram negative bacterium *Histophilus somni* (formerly *Haemophilus somnus*). Alternatively, several pathological syndromes associated with this pathogen were recently identified in cattle from different geographical regions of Brazil. The objectives of this study were to investigate the causes of an acute outbreak of cattle mortality associated with acute neurological manifestations in a feedlot located in Southern Brazil.

Materials and Methods: The owner of a feedlot located in a rural district that is approximately 200 km distant from the city of Londrina, Paraná, Southern Brazil, reported the sudden death of nine yearlings after a brief onset of neurological manifestations. This feedlot acquires cattle for fattening from several geographical regions of Brazil, and at the time had approximately 1200 animals, but the affected yearlings were in a group of 150. Tissue fragments (brainstem, thalamus, cerebrum, cerebellum, and the trigeminal nerve ganglion and the carotid rete mirabile complex [TGN-CT]) from two yearlings (animal 1 and 2) were received in duplicate for pathological and molecular analyses. Animal 1 was euthanized in extremis, while the other yearling died spontaneously. Samples were processed for routine histopathological evaluation and used in PCR and RT-PCR assays designed to detect principal infectious agents associated with neurological disease of cattle from Brazil. These PCR assays targeted specific amplicons of the glycoprotein C gene of bovine herpesvirus type 1 (BoHV-1) and -5, and the 5'-UTR region of the bovine viral diarrhoea virus, the listeriolysin gene of *Listeria monocytogenes*, the 16s rRNA gene of *Histophilus somni*, and the tegument protein gene of ovine herpesvirus type 2 (OvHV-2). Positive and negative controls were included in all PCR and RT-PCR assays. Further, brain samples were submitted for the identification of *Lyssavirus*

Results: Clinically, there was profuse salivation and muscular spasms (animal 1) and motor incoordination (animal 2); the time between the onset of clinical manifestation and euthanasia/death was three days. Further, most neurological manifestations were observed approximately 30 days after arrival of the animals at the feedlot. Significant gross pathological alterations were not observed in the tissues received for pathological analysis from both animals. However, histopathology revealed two distinct neuropathological patterns in several neuroanatomical regions of the brain of both cows: a) moderate multifocal nonsuppurative meningoencephalitis with vasculitis and perivascular cuffings, b) and thrombotic encephalitis with infarction and neutrophilic exudation. These alterations being more pronounced in animal 1, while there was severe perivascular hemorrhage in animal 2. Fibrinoid necrosis was not observed in any tissue evaluated. The BoHV-5 and *H. somni* PCR assays amplified the specific genes of each agent from in both animals: BoHV-5 was successfully amplified from the cerebellum, TGN-CT, and brainstem of both animals and the thalamus of animal #1, while *H. somni* DNA was amplified from the brainstem (animal 1) and thalamus (animal 2). All other PCR/RT-PCR assays yielded negative results, and rabies virus was not identified.

Conclusions: The neuropathological patterns observed in several regions of the brain of these yearlings are consistent with infections due to BoHV-5 (nonsuppurative meningoencephalitis) and *H. somni* (thrombotic encephalitis). Further, the amplification of nucleic acids of these two agents from neurological regions of the brain with histopathological evidence of disease confirmed the participation of these pathogens in these lesions, resulting in coinfections due to BoHV-5 and *H. somni*. Consequently, these infectious agents were associated with the development of the neurological disease outbreak in cattle at this feedlot.

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Internal Medicine

P03-003-125

CASE STUDY: Bovine leukemia virus in a dairy herd of Mexico

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Objectives: Enzootic bovine viral (LVB) is an infectious disease of cattle caused by a lymphotropic virus present in several countries. Courses in chronic infection and asymptomatic form. In seropositive animals is persistent lymphocytosis and occasionally tumors in the uterus, heart, abomasum and lungs. The aim of this study is to confirm the clinical diagnosis of bovine viral leucosis by haematological, molecular and histopathology tests

Materials and Methods: Two clinical case presented is of a cow Holstein Friesian 4 years old, with a history of abortion and low milk production, weight loss, depression, cough and moist rales with presumptive diagnosis of pneumonia. General physical examination was performed finding all normal constant, slightly pale mucous membranes, lymph node chain slightly increased left size and when listening to the lung fields moist rales and cough were found, with a presumptive diagnosis of pneumonia treatment was established based enrofloxacin at a dose of 5-10 mg per kg body weight, once a day for 5 days. However, clinical signs of cough, moist rales, jugular positive pulse palpation and rectal tumors were detected in utero were found changing to a second presumptive clinical diagnosis of viral bovine leukosis. Blood samples were sent to the Diagnostic Laboratory of Pathology of the Agricultural and Industrial Center of Tizayuca, Hidalgo, Mexico, and the Laboratory of Virology, School of Veterinary Medicine (FMVZ) of the UNAM for CBC and insulation LVB virus by PCR.

Results: The results obtained in the above Laboratories, normocytic hypochromic anemia, leukocytosis, lymphocytosis and monocytosis, and LVB virus positive by PCR respectively found. After fifteen days of the clinical presentation of the case, the cow died. At necropsy multicenter metastases heart, lung, rumen, abomasum, intestines and uterus lymphoma was observed. In the histopathological findings they were found with lymph node tissue and encapsulated neoplasm delimited by round neoplastic cells in mesentery, uterus and heart.

Conclusions: You must have a tight control on the purchase of animals and keep them in preventive quarantine in addition to the tests for brucellosis, tuberculosis and other diseases and abortion with LBV, once identified infected cows should separate healthy animals and calves must be fed negative mothers milk. Routine clinical and reproductive management must be performed with hygiene. Furthermore, the reusable material as applicators for insemination, surgical equipment or instruments to dehorning or aretado be disinfected or sterilized before use. Finally it should be insect control and also constitute a transmission path.

Internal Medicine

P03-003-126

Epidemiology And Risk Factors Of Pyelonephritis In Israeli Dairy Cattle

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Objectives: The main objective of the study is to define the risk of pyelonephritis associated with urine sampling via urethral catheterization; the secondary objectives are to describe the current epidemiology and define other risk factors for pyelonephritis in Israeli dairy farms.

Materials and Methods: The research is a retrospective case control study, which was conducted on lactating cows that calved between July 2006 and June 2013 in three commercial herds consisting of 250-450 Israeli Holstein dairy cows. The diagnosis of pyelonephritis was based on clinical manifestation of the disease as reported before. Shortly, all cows went through a comprehensive clinical examination including rectal palpation and urine extraction via urethral catheterization. In part of the cases the urine was sampled using an aseptic catheterization technic, and 10-20 ml urine sample was collected into a sterile 30 ml vial. The urine sample was refrigerated and sent for urine analysis and bacterial culture up to 48 hrs from sampling. Treatment of the animals was initiated at diagnosis and altered, if necessary, according to bacteriological findings and antibiogram. Computerized data were retrieved from the herds and the Israeli herd book, and analyzed using Excel (version 2010, Microsoft, Redmond, WA) and SPSS (version 19, IBM Business analytics, NY USA). Lactation Incidence Risks for all recorded calving diseases and culling data were evaluated for healthy control cows and for pyelonephritis cases. For multivariate analysis two models were built: a multivariate logistic regression using the binary logistic regression function for the general population, and conditional logistic regression using the cox survival analysis function for the case control study. The final models were built with exit criteria set at $P > 0.10$, values of $P < 0.05$ were considered significant.

Results: 74 cows out of 7052 lactations (1.05%) were clinically diagnosed as suffering from pyelonephritis, Most were diagnosed at the beginning of the lactation (40% of all cases were diagnosed during the first 30 days; 60% during the first 90 days of the lactation).

44 urine samples were sent for bacterial analysis.. *C. renale* was isolated only from one sample (prevalence of 2.3%) in combination with *E.coli*. *E.coli* was the most prevalent bacteria in urine samples from cows diagnosed with pyelonephritis (N=30; 27 cases as a sole bacteria and 3 mixed infections; prevalence of 65.9% and 61.4% respectively). The second most prevalent bacteria was *Proteus mirabilis* cultured from 6 samples (13.6%) including one case of mixed infection with *E.coli*. In 8 cases no bacterial growth was demonstrated although bacteria, neutrophils and granular casts were demonstrated in cytology done in 3 cases.

Older cows are at risk for pyelonephritis in relation to 1st calf heifers (OR= 2.381 and OR = 2.891 for 2nd lactation and older cows respectively). Endometritis, Ketosis and twin calving were also identified as risk factors for pyelonephritis (OR =2.927, 1.693 and 2.206 respectively).

Catheterization before the diagnosis date of pyelonephritis was identified as a risk factor using the X² test (OR= 1.995, P=0.017) but when the effects of the factors Number of calves, CM and Ketosis were included catheterization before the diagnosis of pyelonephritis was not associated with UTI (P=0.133).

Conclusions: Pyelonephritis of the Israeli dairy cow is a disease associated with calving diseases (Twin calving, EM and Ketosis) and

higher parity. The most prevalent bacteria isolated from urine of cows with UTI are *E.coli*. Initial treatment of cows with pyelonephritis should be aimed at these bacteria, until bacterial isolation from the urine is obtained. Urethral catheterization after thought cleaning of the perineum with water and soap is not a risk factor for UTI in the dairy cow.

Internal Medicine

P03-003-127

A preliminary descriptive study and meta-analysis of patterns of bovine neoplasia in Ireland.

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Objectives: There are no published reviews of bovine neoplasia in Ireland, although a range of tumours are known to be present, including ocular squamous cell carcinoma (OSCC) which causes economic losses and has implications for animal welfare. Ireland is free from another economically important tumour, enzootic bovine leukosis (EBL), but sporadic bovine lymphomas (SBL) do occur. This preliminary report describes bovine tumours identified from a number of sources. A meta-analysis was performed on the different sets of data using available information on affected animals, to attempt to identify risk factors associated with certain tumours.

Materials and Methods: Three different sets of bovine tumour data were available. Dataset 1 comprised 48 tumours identified at post-mortem inspection in a number of Irish abattoirs from January 2014 to November 2015.

Dataset 2 comprised tumours identified from carcass referrals to the Veterinary Laboratory Service, from January 2010 to November 2015.

Histopathology was performed on formalin-fixed, paraffin-embedded tissue from all tumours in Datasets 1 and 2. Whenever lymphoma was diagnosed, either polymerase chain reaction (PCR) for enzootic bovine leukosis virus (EBLV) was performed directly on fresh-frozen tumour tissue, or an ELISA-based serological investigation was carried out in the herd of origin.

Dataset 3 was observational data on 279 ocular neoplasms (presumptive OSCC) identified at ante-mortem inspection in one abattoir between December 2009 and December 2013. Histopathological confirmation was not performed on the tumours from dataset 3.

County of birth, animal breed, age and gender were available from the Department of Agriculture, Food and the Marine's (DAFM) Animal Health Computer System (AHCS).

Results: Of the 48 tumours in Dataset 1, 28 (58%) were confirmed as OSCC, 6 (13%) as SBL, 5 (10%) as metastatic carcinoma/adenocarcinoma of unidentified origin, with melanomas, fibromas, and nerve sheath tumours also represented. All but five of the animals were female. Mean age at submission was 11.1 years. Commonest breeds were Friesian (Fr), MountBeliarde (Mo), Simmental (Si), Hereford (He), Limousin (Lm) and Other having frequencies 16, 5, 4, 4, 3 and 8 respectively.

In dataset 2, a total of 54 bovine tumours were detected at necropsy, thirty two (59%) of which were sporadic lymphoma.

In dataset 3, all animals were female. Commonest breeds were Fr, Mo, Si, He, Charolais (Ch) and Other having frequencies 136, 47, 38, 38, 7 and 13 respectively. Across all breeds the mean age at detection was 9.7 years with breeds Fr, Mo, Si, He and Ch having mean ages of 9.4, 9.3, 10.6, 9.7 and 11.4 years, respectively. The mean age at detection was significantly different ($p < 0.05$) between breeds Mo (the youngest) and He and Ch (the two oldest).

PCR and ELISA for lymphoma: all negative

Conclusions: This was a preliminary, descriptive study of bovine neoplasia rather than an accurate measure of prevalence.

OSCC was a relatively common tumour detected in abattoirs but not in submissions to the laboratory service, highlighting the value of abattoir surveillance for certain conditions.

There are indications of possible breed effects for OSCC (with Mo, He over-represented). This is probably related to lack of eyelid pigmentation but, in the case of Mo, may warrant further investigation.

Sporadic lymphomas were the most common tumours in submissions to the laboratory service.

Internal Medicine

P03-003-128

PLASMA AND PERITONEAL CONCENTRATIONS OF HMGB1 AND D-DIMER FROM CALVES WITH OR WITHOUT UMBILICAL HERNIA

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Objectives: HMGB1 is a highly conserved protein across species, important in stimulating the release of several chemokines and pro-inflammatory cytokines by monocytes. The D-dimer is a degradation product linked to fibrin from fibrinolysis, strongly associated with hypercoagulability activity. The objectives were to determine plasma and peritoneal concentrations of the HMGB1 and D-dimer proteins in calves with congenital umbilical hernia (CUH) before and after hernia repair, determining which compartment these proteins reflect better and faster inflammatory changes.

Materials and Methods: Were collected peritoneal fluid (PF) and venous blood of 20 Holstein calves with umbilical hernia ($n = 10$) and healthy ($n = 10$) on day 0 (before hernia repair), 1, 3, 5, 7 and 15 days. The HMGB1 levels were determined by sandwich ELISA (ST 51011 kit, IBL International, Toronto, ON, Canada.) as described by Brown et al. (2009), and D-dimer determined using an immunoassay kit (Nycocard Reader D-dimer, Axis-Shield, Shanghai, China) as described by Wittek et al. (2010), comparing the time between samples and between groups.

Results: Congenital umbilical hernia caused elevation of HMGB1 concentrations both in plasma and in the peritoneal fluid (PF) when compared to controls, whereas D-dimer levels were increased in plasma. HMGB1 is an important pro-inflammatory cytokine in various diseases. Because no animal showed ischemia of the herniated portion, no alteration of D-dimer levels were observed locally. After hernia repair, the concentration of HMGB1 decreased in the first five days in plasma and PF, and increased at 15 days after surgery in PF. This cytokine may be as early or late trigger modulators of inflammation depending on the

stimulus. D-dimer remained elevated in the hernia group up to 7 days in plasma and up to 3 days in PF compared to the controls. These findings correspond to a modest increase in systemic and local fibrinolytic activity after repairing a hernia due to the degradation of fibrin.

Conclusions: Increases of HMGB1 and D-dimer levels suggest that the local and systemic acute inflammatory response were caused by the presence of the umbilical hernia in calves. Hernia presence increased D-dimer concentrations previously to HMGB1. Finally, HMGB1 and D-dimer can be used in the evaluation of local and systemic inflammatory response in calves with umbilical hernia.

Internal Medicine

P03-003-129

Use Of Alivecor Heart Monitor For Heart Rate And Rhythm Evaluation In Water Buffalo Calves (Bubalis Bubalis)

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Objectives: Objectives of this study were to evaluate the use of a smartphone-based electrocardiogram (ECG) device (AliveCorECG™) in dairy water buffalo calves for the determination of heart rate and rhythm. Additionally, the quality of ECG from the AliveCor device was compared to a standard 6-lead ECG.

We hypothesized that the AliveCor device could be used to obtain an accurate heart rate and rhythm in water buffalo calves in a field setting.

Materials and Methods: Six healthy neonatal water buffalo calves had simultaneous AliveCor and standard 6-lead ECGs performed. Calves were haltered and recordings were taken in a standing position. The AliveCor ECG was recorded with the device placed over the left cardiac apex. Paper speeds was 25 mm/sec and amplitude was 10 mm/mV. All ECGs were evaluated unpaired and independently by a data-blinded veterinary cardiologist. The average heart rate was determined based on 6 seconds of ECG recording, rhythm diagnosis was recorded for each tracing, and each ECG was given a quality score. The findings were then reviewed to determine if the AliveCor device was comparable to the 6 lead ECG for rate and rhythm diagnosis. A paired t-test was used to assess for significant differences between each calf's average heart rate determined by the AliveCor device and the 6-lead ECG, and a Mann-Whitney test was used to compare the quality scores between the two readings for each calf.

Results: All animals were healthy and had no murmurs on pre-recording physical examination.

There was no significant difference between average heart rates obtained between AliveCor recordings when compared to those from the 6 lead ECG ($P = 0.174$). A normal sinus rhythm was recorded for all 12 recordings.

The AliveCor recordings of 4/6 calves most closely resembled lead aVR, while recordings of 2/6 calves more closely resembled lead II.

No significant difference was observed between standard and AliveCor quality scores ($P=0.6250$).

Conclusions: We conclude that the AliveCor device allows for accurate heart rate determination and diagnosis of a normal sinus rhythm in neonatal dairy water buffalo calves, when taken in a standing position from the left heart base in a field setting.

Internal Medicine

P03-003-130

Insulinoma In A 5-Year-Old Dexter Cow With Persistent Hypoglycemia

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Objectives: A Dexter cow aged five years in the fifth month of pregnancy was referred to the Clinic for Ruminants and Swine, Department of Veterinary Medicine, Freie Universität Berlin, Germany, by a local practitioner due to general weakness and ataxia that did not respond to treatment.

Materials and Methods: At admission, the cow was clinically examined according to Rosenberger [1] and blood samples were obtained for laboratory testing. An intravenous glucose tolerance test was performed. While plasma glucose concentrations were determined in the laboratory of the clinic, serum samples for determination of insulin were sent to the Endocrinology Laboratory, Clinic for Cattle, Tierärztliche Hochschule Hannover. A radioimmunoassay was applied for the quantification of serum insulin concentrations in cattle¹.

Results: The general condition and demeanor of the cow were severely impaired. The cow demonstrated an uncertain stance characterized by inability to properly support the body weight by its legs. When walking, the animal showed ataxic movements and a hypermetric gait. Besides the symptoms described above, the neurological examination demonstrated bilateral mydriasis as well as delayed pupillary reflexes (direct/indirect).

Results of blood analysis revealed a persistent hypoglycemia (glucose levels: 1.4 – 1.9 mmol/l). The Dexter cow, however, did not respond with ketone body production or excessive lipomobilization in the face of hypoglycemia. Therefore, an insulin-secreting tumor was suspected and an intravenous glucose tolerance test (IVGTT) confirmed the latter assumption.

Conclusions: All treatment attempts including providing glucogenic nutrients and precursors (glycerol and propylene glycol) failed and the condition of the cow deteriorated during hospitalization. Due to the clinical condition and the unfavourable prognosis, euthanasia was elected and the animal was sent for necropsy to the Institute of Veterinary Pathology, Faculty of Veterinary Medicine (Freie Universität Berlin). At necropsy, multifocal, highly infiltrative, white-grey nodules were present in the pancreas. An immunohistochemical examination confirmed the clinical diagnosis of insulin-producing islet cell tumor in the pancreas.

Comments: 1. Rosenberger, G. Clinical examination of cattle. Paul Parey, Berlin and Hamburg. Germany. 1979.

¹ IM3210, insulin IRMA KIT, Immunotech, Beckman Coulter, CA, USA

Internal Medicine

P03-003-131

Bacterial Communities Associated With Healthy Abomasal Mucosa And Ulcers In Cattle

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Objectives: The present study was performed to characterize bacterial communities of slaughter cows, bulls and calves. The communities of healthy and ulcerated mucosa were compared to potentially identify pathogens contributing to the lesions.

Materials and Methods: Abomasal mucosa samples were taken after slaughter from three different groups: fattening bulls, cows, and calves. Within each group, the ulcerated abomasal mucosa of affected animals, unaffected mucosa of these same animals with ulcers, and abomasal mucosa of healthy animals were sampled. In total, 63 samples from 42 animals were analyzed. DNA of the samples was extracted and the V1–V2 region of bacterial 16S rRNA genes was amplified and pyrosequencing was performed. The sequences were analyzed using the software mothur and classified into operational taxonomic units (OTUs). Furthermore, diversity indices and Yue & Clayton Theta similarity coefficients were calculated. The similarity between bacterial communities was analysed and it was determined which OTUs showed a statistically significant different abundance between the groups.

Results: The bacterial communities associated with abomasal ulcers of slaughter cows, bulls, and calves in Austria were described using 16S rRNA gene pyrosequencing. Sequences were clustered into 10,459 operational taxonomic units (OTUs), affiliating to 28 phyla with Proteobacteria, Firmicutes, Bacteroidetes and Tenericutes dominating (96.4% of all reads). The most abundant genera belonged to Helicobacter, Acetobacter, Lactobacillus, and novel Mycoplasma-like phylotypes. Significant differences between the microbial communities of calves compared to cows and bulls could be observed. However, only few statistically significant differences in the abundances of certain OTUs between healthy and ulcerated abomasal mucosa were found.

In all groups there was a high diversity of bacteria attached to the abomasal mucosa. When comparing ulcerated mucosa, unaffected tissue of the animals with ulcerated mucosa and mucosa from healthy animals no statistically significant differences of species richness and diversity estimators were found. Only significant differences for the different animal groups (cows, bulls, calves) and also when comparing adult animals (cows and bulls) and calves were found.

Conclusions: The present study provides the first deep insights into the bacterial microbiota of healthy and ulcerated abomasal mucosa in cattle. A high diversity of bacteria attached to the abomasal mucosa in bulls, cows and calves was found. Distinct differences in the bacterial communities between calves on one hand and cows and bulls on the other hand have been identified. A relationship between microbiota and abomasal ulcers could not be established. However, since all ulcers in cattle of the study were least severe type of ulcers the question remains if there could be an influence of certain bacteria on the formation of more severe lesions.

Internal Medicine

P03-003-132

Echocardiographic assessment of left ventricular systolic function in calves with naturally occurring severe sepsis and septic shock

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Objectives: Until now, there is no literature information about longitudinal transthoracic echocardiographic (TTE) evaluation of cardiovascular insufficiency and changes in left ventricular systolic function in calves with naturally occurring severe sepsis and septic shock and how systolic function is affected by fluid therapy in calves. For this reason, the aim of the study was (1) to evaluate changes in left ventricular systolic function by TTE before and after treatment and, (2) to evaluate the echocardiographic changes in LV systolic function according to the treatment options.

Materials and Methods: Ten hypotensive calves, 0-4 weekly, with complete laboratory and clinical data necessary for systemic inflammatory response syndrome and multiple organ dysfunction syndrome classification (experimental group), and 5 healthy calves, 0-4 weekly, (control group) were used as materials. TTE examinations were performed before the treatment, after the first bolus of fluid resuscitation and on the first, second and third days of the treatment. To assess LV systolic function, end diastolic volume index (EDVI), end systolic volume index (ESVI), ejection fraction (EF%), fractional shortening (FS%), stroke volume (SV), cardiac index (CI), pre-ejection period/ejection time (PEP/ET) were determined in the experimental group of calves. TTE examinations for the determination of the same systolic function indices were performed once on the control group of calves. Non-invasive arterial blood pressures [Systolic blood pressure (SBP) and mean arterial pressure (MAP)] were also monitored during the hospitalization. Fluid therapy [boluses containing hydroxyethyl starch (10 ml/kg/h) and 0.9 % NaCl (65ml/kg/h)] was administrated to the experimental group of calves during the hospitalization. The goal in the fluid therapy of the experimental group of calves was to obtain the followings; systolic blood pressure >90 mm/Hg and/or mean arterial pressure >65 mmHg, normalisation of clinical and cardiological findings, and lactate levels <4 mmol/L.

Results: SBP and MAP (71.9±6.53 mmHg and 51.7±3.96 mmHg, respectively) were low at the admission and increased after the first bolus of fluid resuscitation (123±5.76 mmHg and 88.1±3.81 mmHg, respectively) (p<0.05) and remained normal during the hospitalization (p>0.05). EDVI and ESVI (15.3±3.58 ml/m² and 6±1.91 ml/m², respectively) increased after the first bolus of fluid resuscitation (44.8±4.44 ml/m², 20±2.85 ml/m², respectively) (p<0.05) and did not change during the hospitalization (p>0.05). SV and CI (9.2±1.48 ml and 1150±247 ml/min/m², respectively) increased after the first bolus of fluid resuscitation (24.9±2.52 ml and 3165±45 ml/min/m², respectively) (p<0.05) and did not change during the hospitalization (p>0.05). The EF, FS and PEP/ET did not different during the study (p>0.05).

Conclusions: The result of the study showed that fluid resuscitation in calves with severe sepsis and septic shock improved the preload and the afterload and TTE was a very useful tool in the monitorization of fluid therapy and it's effect on cardiovascular system.

Internal Medicine

P03-003-133

Effect of sedation on the intrapulmonary position of a bronchoalveolar lavage catheter in calves

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Objectives: The vast majority of bacterial pneumonias in calves are situated in the cranioventral lung lobes. Non-endoscopic sampling with a broncho-alveolar lavage (BAL) catheter is a practical method to sample larger groups of animals in an economic way. For practical and welfare reasons sedation of calves during the BAL procedure might be warranted. To date it is unknown which lung lobes are sampled by this non-endoscopic procedure and whether this is influenced by sedation. Therefore, the objective of this study was to determine the effect of the most commonly used sedation in cattle (xylazine) on intrapulmonary position of a BAL catheter.

Materials and Methods: A randomised clinical trial was performed on 18 male Holstein Friesian calves (mean age=8.5 months +- standard deviation (SD)=3.5 months). Calves were randomised over the two treatments, being sedation with a single dose of 0.1 mg/kg body weight xylazine intramuscularly (Xyl-M 2%, VMD, Arendonk, Belgium) and a placebo (2 mL of saline solution). In each calf a BAL was performed. The intrapulmonary position of the BAL catheter was determined by endoscopy and contrast-radiography with barium. In the control group this positioning was validated by euthanasia with the catheter in place and post-mortem examination. Histological examination was done for 3 calves at different sites of the respiratory tract (nasopharynx, trachea, large bronchi and the different lobi). For statistical analysis the intrapulmonary position of the catheter was characterised with binary values (cranial vs. caudal; left vs. right and dorsal vs. ventral) and logistic regression was used.

Results: A BAL catheter was introduced in 18 calves, of which 11 were sedated and 7 served as controls. In the control the probability of sampling the different lung parts is equally distributed in the control animals: 14.3% dorsocranial, 28.6% dorsocaudal, 28.6% ventrocranial and 28.6% ventrocaudal. In contrast, in sedated animals systematically the dorsocaudal lung part was sampled (100% vs. 28.6%). Sedation had a significant effect on the dorsal vs. ventral (P<0.05) and cranial vs. caudal positioning (P<0.05), but not on left-right distribution (P= 0.49). Histology revealed acute inflammation of the proximal nasal cavity, proximal trachea and distal trachea.

Conclusions: In conclusion, a BAL-catheter, introduced without endoscopic guidance, samples a random lung lobe in unsedated animals, and not systematically the most affected cranial lobes. When sedation is applied, the dorsocaudal lung lobes, which are less frequently affected, are systematically sampled. To what extend this difference in intrapulmonary position of the BAL-catheter affects the likelihood to isolate the causative bacteria remains to be determined.

Internal Medicine

P03-003-134

Early Signs Of Diarrhea In Neonatal Calves In The First Fifteen DaysOnur Topal¹ Hasan Batmaz^{1*}¹Internal Medicine, University of Uludag, Faculty of Veterinary Medicine, Nilüfer-Bursa, Turkey

Objectives: Health of neonatal calves is very important. Therefore, preventive measures and early treatment of diseases are essential to prevent economic losses. The objective of the study was to investigate daily clinical signs in the first 15 days in neonatal calves whether clinical manifestations of the diarrhea.

Materials and Methods: Clinical investigations were performed in 52 Holstein calves. Calves were examined on each day in the first 15 days after birth. In these examination, posture, enophthalmus, skin elasticity, capillary filling time (CFT), appearance of eyes, lacrimation, colour of conjunctiva and mucosa, appearance of muzzle, temperature of muzzle and tip of nose, nasal discharge, sucking reflex, palpebral reflex, temperature of mouth, movements of ears, position of ears, temperature of ears, heart frequency, auscultation of heart, respiration frequency, auscultation of lung, cough, rectal temperature, volume, consistency, smell and colour of feces, lethargy score of the calves were evaluated totally 27 parameters on each day. All parameters were evaluated as normal (score 1), questionable healthy (score 2) and disease sign (score 3). It was observed that 18 calves were healthy and 34 calves had undifferentiated diarrhea.

Results: A day before these 34 calves suffered diarrhea, most observed score 2 and score 3 parameters, respectively, were as follows: Increased heart frequency (24, 70.5%), softening of consistency and bad smell of feces (16, 47.5%), coldness of ears (14, 41.17%), yellow-gray colour feces (13, 38.23%), change in feces volume (11, 32.25%), CFT 2-3 sn (10, 29.41%), change in conjunctiva and mucosa colour, and lacrimation (9, 26.47%), abnormal posture (low shoulders, kiphotic and upturned tail), enophthalmus and lethargy score 2 (8, 23.52%), skin losses its elasticity 2-4 sn and nasal discharge (7, 20.58%), abnormal rectal temperature, coldness muzzle and tip of nose, and weakening of sucking reflex (6, 17.64%). Average birth weights of calves suffered from diarrhea was 39.32 kg, others was 40.19 kg. Average weight on 15th day was 41.47 kg and 43.75, respectively. Although those calves not suffered from diarrhea gained approximately 2 more kg than the ones with diarrhea, it wasn't statistically significant.

The bad smell and yellow-gray color of feces, coldness of ears, prolonged of CFT, change of conjunctiva and mucosa colour, lacrimation, posture, enophthalmus and lethargy were observed as early symptoms occurs before diarrhea in neonatal calves. Approximately %70 of calves had increased heart frequency. However this might be result of stimulation of the calves. Diarrhea occurs before softening to the consistency of the feces are expected to be finding.

Conclusions: As a result, the first 15 days of neonatal calves the bad smell and yellow-gray color of feces, coldness of ears prolonged of CFT, change of conjunctiva and mucosa colour, lacrimation, posture, enophthalmus and lethargy, it can be argued that as early signs occurs before diarrhea.

Internal Medicine

P03-003-135

Outbreak of hiperacute highly lethal faringeal flegmon in a Limousin herd originated by a new Pasteurella multocida, type ST 63Juan V González-Martín^{1,2*} Raquel Patrón¹ Juan A. Díaz de Tuesta³ Natividad Pérez-Villalobos¹ Cristiana Justo¹ Ana I. Vela²¹Trialvet SL, Cabanillas de la Sierra, ²Universidad Complutense, ³Laboratorio Regional de Sanidad Animal, Madrid, Spain

Objectives: Description of an outbreak of pharyngeal flegmon with very high mortality, by Pasteurella multocida, in an extensive herd of 40 Limousin cows, 1 bull, 10 heifers and 34 calves, in the province of Toledo (central Spain) from 11th to 23th April 2015 and typing of bacteria.

Materials and Methods: An epidemiological study was conducted. Necropsies were performed on 2 calves, 2 heifers and 1 cow. Samples were taken for standard histopathological and microbiological study. Presumptive identification of isolate as P. multocida was performed by Gram staining, oxidase and catalase test and the commercial identification system API 20NE test strips (bioMérieux), according to the manufacturer's instructions. Capsular types of the isolate (A, B, D, E, and F) were determined using multiplex PCR (Townsend et al., 2001). The gene toxA, which encodes the dermonecrotic toxin of P. multocida, was determined as previously described (Lichtensteiger et al., 1996). Multi locus sequence typing (MLST) was carried out as previously described (Davies et al., 2003). MLST alleles and the resulting sequence type (STs) were assigned through submission of the respective data to the multihost P. multocida MLST database (http://pubmlst.org/pmultocida_multihost/). The antimicrobial susceptibility was tested by the diffusion method as described by the Clinical Laboratory Standards Institute (CLSI, 2015). The animals were treated with oxytetracycline LA im and vaccinated with a poliostridial vaccine.

Results: Within 13 days of the outbreak 4 cows (10%), 2 heifers (20%) and 9 calves (26.5%) died, with a course of 24 hours. Only 1 cow showed throat inflammation at mass treatment time survived without sequels, all the others affected animals were found dead or died immediately after displaying severe respiratory distress without depression. The outbreak ceased when all animals were prophylactic treated, without cases out the dates indicated. All necropsied animals and the remainder observed by the owner presented exclusively inflammation with extensive oedema in the throat area other than a heifer presented the problem in a limb and died in three days. Only flegmon was found in autopsies and the cause of death was asphyxiation. Purulent laryngitis was diagnosed in the pathological study of 3 sampled animals. Gram-negative rod-shaped organisms about 1 µ and neutrophil infiltration, oedema, hemorrhage, vasculitis, thrombosis and microabscesses was observed in all tissues around the pharynx: salivary gland, connective tissue, muscle and retropharyngeal lymph. P. multocida was isolated in pure culture in the retropharyngeal lymph of the only 2 animals sampled, and was characterized in the capsular type B. The dermonecrotic toxin gene toxA was not detected. With the multi host P. multocida MLST database, the isolate was assigned to the new sequence type ST63. In the antibiogram the isolate was susceptible to tetracycline, florfenicol, ampicillin, gentamicin, ceftiofur, ST and enrofloxacin; resistant to penicillin and clindamycin; and intermediate to erythromycin, and amikacin.

Conclusions: P. multocida is a Gram-negative bacterium. Capsular type A is a common bovine nasopharyngeal commensal and a pathogen implicated in BRD and in lesser extent mastitis and vaginal discharge. Types B and E are implicated in hemorrhagic septicemia (HS) not confirmed in Western Europe. And type F in head oedema. None of the necropsied animals were septicemic and not showed multisystemic petechial haemorrhages or depression characteristics of HS. A hard and

spiny hay offered just before the outbreak started could have traumatized the pharynx and being the potential gateway of this novel clinical picture originated by a new *P. multocida* type ST63.

Internal Medicine

P03-003-137

Intravenous electrolyte solution with high concentration of sodium lactate can correct metabolic acidosis in goats with acute rumen lactic acidosis

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Objectives: The aim of this work was to test the efficacy of an intravenous electrolyte solution formulated with high concentration of sodium lactate for the correction of metabolic acidosis in goats with induced acute rumen lactic acidosis (ARLA).

Materials and Methods: Six healthy saanen goats weighing 40kg body weight (BW) were induced to acute rumen lactic acidosis with 15g of sucrose per body kg. Each goat was submitted to induction twice, with at least 30 days of intervals. The goats were allotted to two groups where treatment consisted in continuous intravenous infusion of 4L (25mL/Kg/h) of an electrolyte solution containing 84mEq/L of sodium lactate (L84) or 84mEq/L of sodium bicarbonate (B84). Blood and urine samples were taken at times -18h (before the induction), 0h (start of infusion), 2h (middle of infusion), 4h (end of infusion), 6h, 12h and 24h after the beginning of infusion. Blood gases, pH, electrolytes and L-lactate were measured. The anion gap (AG) and strong ion difference (SID) were calculated. The data were analyzed by two way repeated measures ANOVA.

Results: Clinical signs and ruminal fluid alterations compatible with ARLA were observed in all animals 18 hours after the induction. The metabolic acidosis due to ARLA was moderate with a reduced blood pH (L84 group: 7,275±0,08; B84 group: 7,232±0,08), decreased HCO₃⁻ (L84 group: 16,03±2,27mmol/L; B84 group:14,40±2,27mmol/L) and BE (L84 group:-11,56±3,23mmol/L; B84 group:-13,33±3,92mmol/L). Goats that received L84 presented the following values at the end of the infusion: pH=7,471±0,07; HCO₃⁻= 26,88±2,30mmol/L; pCO₂=36,58±5,24mmHg; BE=3,71±3,19mmol/L; Na⁺=142,88±2,65mmol/L; K⁺=3,00±0,93mmol/L; Cl⁻= 102,96±3,74mmol/L; SID= 42,92±1,49mmol/L and AG=16,13±1,76mmol/L. The values in B84 group were similar to these. Both treatments were able to correct the metabolic acidosis, dehydration and electrolytes imbalances presented without differences between them (p>0,05). The plasmatic L-lactate concentration increased during the infusion of L84 solution from 2,03±0,69mmol/L in the beginning to 9,35±1,28mmol/L at the end. However, returned to low levels six hours later (1,0±0,67mmol/L). The L-lactate fractional clearance increased only in the end of the L84 infusion, indicating that L-lactate was metabolized and the excess was excreted. All goats were completely recovered 3 to 4 days later.

Conclusions: The novel electrolyte solution containing high concentration of sodium lactate (L84) was effective in the treatment of metabolic acidosis induced by ARLA in goats, as an alternative to

bicarbonate solutions. Infusions of large volumes are safe and no side effects were observed.

Internal Medicine

P03-003-138

The effect of current standard therapy on clinic, hematologic and biochemical parameters in calves with severe sepsis and septic shock

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Objectives: Severe sepsis and septic shock that cause high mortality rate are commonly seen in both human and veterinary medicine. Until now, unfortunately, there is no longitudinal clinical research concerning with treatment of calves with naturally occurring severe sepsis and septic shock.

The aim of this study was to evaluate the effectivity of current standard therapy (oxygen, antibiotic and fluid administration) on clinic, hematologic and biochemical parameters during the treatment of calves with naturally occurring severe sepsis and septic shock.

Materials and Methods: Ten hypotensive calves, 0-4 weekly, with complete laboratory and clinical data necessary for systemic inflammatory response syndrome and multiple organ dysfunction syndrome classification (experimental group), and 5 healthy calves, 0-4 weekly, (control group) were used as materials. Blood samples for hematology, blood gas and serum biochemistry were collected once from the control calves. However, blood samples were collected before treatment and after treatment as intervals from vena jugularis in the experimental group calves. As treatment, oxygen, antibiotic (ceftiofur, 2.2 mg/kg) and fluid were administrated. The experimental calves were monitorized by ECG during treatment.

Results: According to the history taken from the owners, the calves had been affected about 24 hours and over. Calves had no suckling reflex, mostly lateral in position and mental depression. Clinical findings of calves did not improve after the treatment at 72 hours. There was no statistically difference in clinical findings between baseline and after treatment. All the calves had severe metabolic acidosis (pH<7.2) and > 8 dehydration degree at admission. Each calf received intravenous colloid (HES 10 ml/kg/h)+crystalloid (0,09%NaCl 65ml/kg/h) solution combination during the treatment. Venous blood pH was increased (pH>7.3), but lactate levels (<2.3 mmol/L) was decreased after the treatment at 6 h. Most of the calves had high whole blood count (WBC) on admission. However, WBC was decreased after treatment at 72 hours, but WBC was still high compared to control group. Serum creatinine, AST, ALP and GGT levels of the experimental group calves were still high after the treatment at 72h compared to the values before treatment (p<0.05).

Conclusions: Current standard therapy in calves with naturally occurring severe sepsis and septic shock improved acid-base parameters and lactate levels, but not the clinical findings. It was considered that majority of the calves might have died from multiple organ dysfunction syndrome due to increased serum enzyme levels. Therefore, it can be concluded that duration of disease in calf with severe sepsis and septic shock is the most important factor for prognosis.

Internal Medicine

P03-003-139

Gas Gangrene caused by *Clostridium Novyi* in a high-end genetic Holstein embryo donor during a series of superovulation injections

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Objectives: A clinical case of gas gangrene caused by *Clostridium Novyi* is presented following multiple injections using the Follotropin V protocol. This was a first calf HF cow that had developed a very firm swelling of the right gluteal region 8 hours post administration of 4th im injection of FSH with sterile needles. The cow was moderately painful and toe-touching lame of the right hind. She was 160 days in milk and ranked in the 99% percentile for milk production and milk fat/protein and was producing 45-50 kg milk/day. Using this case best treatment options and prevention for gas gangrene in valuable Bovine embryo donors is discussed.

Materials and Methods: The cow was from a 700 head free stall dairy herd, milking 3 times daily with TMR feeding. She was subjected to a embryo superovulation procedure using the Follotropin-V protocol. Vaccination of the herd is a 10 way modified live vaccine (BoviShield Gold FP5 + L5) twice pre-breeding and 21-50 days pp with no clostridial vaccines. The cow was alert and responsive and swelling extended from the tuber coxae to ischiatic tuberosity and lumbar spine through to the biceps femoris with no crepitus present. She had received Pen IM once prior to presentation. Vital parameters were normal. Rumen, Udder and CMT, as well as GI tract were normal. Ultrasonography of right gluteal region revealed fluid pouches with no gas but tissue edema. A CBC had neutrophil toxicity while the serum biochemistry showed moderate elevations in AST (248 IU/L) and CK (1768 IU/L and no other abnormalities. Gram stain of a fine needle aspirate of the right gluteal muscles yielded gram positive bacilli and degenerate neutrophils; culture identified +++ *Clostridium novyi*. To relieve tissue pressure and to enable exposure to air a total of 6 vertical fasciotomy incisions of affected gluteal and biceps femoris region were placed along both skin and fascial planes. IV ringers with antibiotic (Na-Pen 44000 IU/kg q6h) and analgesic medication (Flunixin Meglumine 1mg/Kg q12h) were started. Local rinsing with H₂O₂ solution and local hydrotherapy were performed q6h for 30 minutes and she was walked for good blood circulation in affected tissues.

Results: Within hours following fasciotomy, the right gluteal swelling began to decrease; the lameness improved markedly however there was minimal improvement of general comfort level. On day two of hospitalization an epidural catheter was placed and a morphin/detomidine/saline combination was intraepidurally given every 6 h as adjunct analgesia for two days.

The fasciotomy sites were debrided on a daily basis and rinsed with H₂O₂. Warm/cold hydrotherapy and exercise was increased. Over the following three days the cow remained stable but still extremely painful over the affected muscle area. Based on recent information on day three systemic treatment with oxytetracycline (20mg/kg diluted in 1L saline) q24h was added. Milk production had dropped to 0L but gradually returned to >30 L. Medication was discontinued on day 8 after admission. The cow was sent home with the recommendation to vaccinate cows entering the embryo superovulation program with a multiway clostridial vaccine. Over a period of the next two years the cow was kept as an embryo donor. No further cases of gas gangrene were observed in this dairy herd over this period.

Conclusions: Take home message is to re introduce a multiway clostridial vaccine in dairy cows used for embryo superovulation procedures. Dormant spores in healthy musculature of such cows could have preexisted. Presence of dormant spores of a multitude of clostridial species was confirmed in horses in 20% of sterily obtained muscle samples in freshly euthanized horses. Unpublished data from our lab showed that 5% of such muscle samples in cattle were positive for organisms such as *C. perfringens* and *C. difficile* among others. Early treatment of clostridial myonecrosis with tissue fasciotomy might be the best way to have successful outcome of such cases.

Internal Medicine

P03-003-140

Blood Gas Analysis In Relation To Clinical Symptoms From Diarrhea Calves

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Objectives: Calf diarrhea is a major cause of economic loss to cattle industry worldwide. The aims of this study were to determine blood gas and biochemical values and to analysis blood values in relation to clinical symptoms from diarrhea calves.

Materials and Methods: From July to September in 2014, a total of 68 diarrheal calves under 3 months old (both Korean native calf and Holstein) were investigated. History taking and physical examination were conducted. The depression score of physical activity was classified as bright, dull, dummy, and coma. The venous blood sample was collected from jugular vein and immediately analyzed by iSTAT 1 portable clinical analyzer (Abbott Point of Care Inc., Abbott Park, IL 60064, USA) with the EC8⁺ cartridge. The relationship between the clinical symptoms and blood values was analyzed by Jonckheere Terpstra trend test.

Results: In 68 diarrheal calves, 8, 22, 34, and 4 calves belonged to bright, dull, dummy, and coma, respectively. The values for pH, carbon dioxide partial pressure, bicarbonate, base excess, and glucose were decreased according to deterioration of physical activity status ($p < 0.05$). And the values for anion gap, hemoglobin, hematocrit, and potassium, BUN were increased getting with deterioration of physical activity status ($p < 0.05$). The values of hematocrit ($p = 0.006$), hemoglobin ($p = 0.007$), and total carbon dioxide ($p = 0.002$) were good prognosis indicator. The value of BUN according to physical activity status was significantly increased getting with deterioration of physical activity status ($p < 0.0001$). The difference in concentration of sodium and chloride among each physical status groups were not statistically significant (p value > 0.05).

Conclusions: This study provides the information on the blood gas status of diarrhea calves in summer season and guide practitioners through a decision-making process which will minimized the negative impact of diarrhea on individual patients as an accurate diagnosis and ultimately its economic impact on the cattle industry as a whole.

Internal Medicine

P03-003-141

Arterial And Venous Blood Gas, Electrolytes, Biochemical And Hematological Values In Healthy Korean Native Calves

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Objectives: The normal reference data on blood gas analysis of Korean native cattle has not been examined yet. The objective of this study was to investigate arterial and venous blood gas figures of Korean native calves. We studied 62 calves under 3 weeks old to find reference data of Korean native calves. Furthermore, we compared arterial blood gas data with those of venous blood.

Materials and Methods: The 62 healthy Korean native calves under 3 weeks old were examined. The arterial blood was collected from caudal auricular artery (Fisher and others 1980) and the venous blood from jugular vein. The blood samples were analysed immediately using a portable blood gas analyser (EPOC, Woody Equipment Company Ltd, UK). The pH, pO₂, pCO₂, cHCO₃⁻, BE, cSO₂, Na⁺, Ca²⁺, Cl⁻, aniongap potassium(AgapK), Hct, cHgb, glutamine, lactate and creatinine were determined. Statistic analysis was conducted with SAS.

Results: The mean of arterial and venous pH, BE, cHCO₃⁻ of 62 Korean native calves were 7.44±0.04 vs 7.41±0.04, 2.3±3.3 vs 4.3±2.9mEq/L and 26.4±2.8 vs 29.0±2.5mmol/L, respectively. Glucose (r=0.927) had the strongest correlations between arterial and venous values. The correlation between the values of the arterial and the venous blood was strong in creatinine (r=0.925), lactate (r=0.815), Ca²⁺ (r=0.806), Hct (r=0.799), Na⁺ (r=0.790), cHgb (r=0.786), base excess (r=0.749), pH (r=0.710), HCO₃⁻ (r=0.710), and cTCO₂ (0.663).

Conclusions: This study obtained the normal reference data of arterial and venous blood gas analysis of Korean native calves under 3 weeks old. The arterial and venous pH, BE, and cHCO₃⁻ values were significantly correlated, respectively.

clinically normal, the experiment was initiated. This experimental study was divided in two phases. The first phase lasted 48 hours. This was the control period and the animals served as their own control. The second phase was the metabolic alkalosis induction period brought about by opening the duodenal cannula to let the abomasal content flow out of the body until inducing metabolic hypochloremic alkalosis determined in blood using the iStat® blood gases analyzer. Urine samples were taken every 24 hours in both phases.

In the urine several variables were assessed such as: pH was measured using a Schoot Lab 8500 potentiometer, net acid excretion (NAE) by the Jørgensen's method that allows to calculate ammonium content and titrable acidity in urine. SID and urinary pH were calculated according to the method proposed by Constable et al (2009) where SID = NBE (Net Base Excretion)+ 2,6 = -NAE + 2,6 and $pH = \log_{10} \{ 2 ([SID] + [NH_4]) / \{ (K_1' S P_{CO_2} + K_{a_2} [PO_4] - K_{a_2} ([SID] + [NH_4]) + (K_1' S P_{CO_2} + K_{a_2} ([SID] + [NH_4] + K_{a_2} [PO_4])^2 - 4 K_{a_2}^2 ([SID] + [NH_4]) [PO_4])^{1/2} \} \}$. The statistical analysis consisted of determining the degree of correlation between SID and urinary pH (measured and calculated ones; and between calculated and measured pH using Pearson's coefficient (r) at a significance level of p<0.01.

Results: It was found in the urine of sheep with experimental induced metabolic hypochloremic alkalosis, that there was a strong correlation between the measured pH and calculated pH (r= 0.847). It was also determined a strong correlation between calculated pH and SID (r=0.839). However, the correlation observed between the calculated urine SID and the measured urine pH was moderate (r=0.588). It was determined that when the experimental subjects develop paradoxical aciduria, there was an important decrease in ammonium concentration that varied from a mean range level of 1.750-2.667 mmol/L during the control phase to 4.667 to 16.250 mmol/L and the urine calculated SID showed also a marked decrease, it went from a range of 133,671 -208.779 mmol/L during the control period to 98.433 to -7.900 mmol/L during paradoxical aciduria.

Conclusions: There is a high correlation between the calculated urine pH using the formula based on SID and measured pH, the SID and calculated pH along with a moderate correlation between measured pH and calculated SID. This indicates that Urinary pH is strongly dependent of SID and pH reduction can be explained by a decrease in the SID.

Comments: The authors declare no conflict of interest

Internal Medicine

P03-003-142

Physico-Chemical Approach To Urinary Acid-Base Alterations In Sheep With Induced Metabolic Hypochloremic Alkalosis

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Objectives: The objective of this study was to determine the relationship of the independent variable strong ion difference (SID) and urinary pH in sheep with induced hypochloremic metabolic alkalosis, using the net acid secretion to calculate the SID in an experimental model of massive loss of abomasal fluid through a duodenal cannula.

Materials and Methods: Seven ovine subjects were surgically implanted with a duodenal cannula. They had a period of recovery from surgery that lasted 14 days. Once, the experimental subjects were determined to be

Internal Medicine

P03-003-143

Fungal rhinitis associated with tracheal collapse in a cow

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Objectives: The incidence of respiratory diseases in cattle is high and there are several pathological agents of respiratory disease in this specie. In some cases, complementary exams such as endoscopy and microbiological culture may be necessary for the specific diagnosis of

these diseases. The aim of this study was to report a case of fungal rhinitis associated with tracheal collapse in a cow.

Materials and Methods: A seven-year-old Holstein cow was referred to the Teaching Veterinary Hospital of the FCAV – Jaboticabal, campus of the Univ. Estadual Paulista “Júlio de Mesquita Filho” - Unesp, with severe inspiratory dyspnea and orthopneic position. Physical exam revealed tachycardia (60 beats per minute), tachypnea (26 respiration per minute), pale mucous membranes and fever (40.9°C). Pulmonary auscultation revealed bilateral crackles sounds and the owner report a previous treatment for pneumonia with penicillin. Antimicrobial therapy was instituted with enrofloxacin (4mg/kg), for 14 days, every 24 hours, with slight clinical improvement. Then an endoscopy was realized and showed redness, edema and fibrinonecrotic lesions on the nasal choanae, with reduction of the nasal lumen and tracheal collapse. A nasal swab was realized for microbiological culture.

Results: Due to the appearance of lesions and lack of resolution of the case with antibiotics, we suspected of fungal infection and introduced a treatment with 20 % solution of sodium iodide (66mg/kg), every 15 days, until clinical improvement, totalizing five administrations. The fungal culture was negative, but the antifungal treatment was maintained due to clinical improvement. The swab result was positive to *Streptococcus* sp. and *Staphylococcus* sp, but the clinical signs did not justify this result. At the end of the treatment, another endoscopy was performed and showed significant improvement of lesions, including tracheal collapse.

Conclusions: The endoscopy exam was essential to determinate the exact location and magnitude of injuries, enabling the monitoring of treatment and evaluate their effectiveness. It also allowed the prognosis evaluation, since the tracheal collapse explain the slight breathing difficult that animal still presents and probably will follow for the rest of the animal's life.

Internal Medicine

P03-003-144

Direct magnesium hydroxide abomasal infusion for ulcer treatment in cow

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Objectives: Metabolic diseases, lactational stress and nutritional deficiencies are commonly observed in dairy cattle. These disorders are associated with greater digestive capacity and corporal depth due to continuous genetic selection for high milk production, leading to susceptibility for left and right-side abomasal displacement, abomasal ulcers and impactations. The aim of this study was to report a case of abomasal ulcers treated with infusion of magnesium hydroxide directly into the abomasum.

Materials and Methods: A two-year-old Holstein-Friesian cow was referred to Centro Universitário Barão de Mauá Veterinary Hospital with inappetence and marked drop in milk production. Upon physical examination, ruminal movements were present but decreased in frequency and intensity and there was a characteristic tympanic resonance (“ping”) during percussion on the left side of the abdomen, dorsally. The animal also had melena and pale mucous membranes and laboratorial findings included slight decrease in red cell count ($4.6 \times 10^6/$

μ l), featuring left-side displacement of the abomasum and perforating abomasal ulcers.

Results: Surgical treatment was elected through abomasopexy by left flank laparotomy. After surgery, two administrations of magnesium hydroxide solution were performed directly into the abomasum (300 g/450 kg BW weight), with a 4-day interval. A spine needle (20 GA, 20 cm) was used to infuse magnesium hydroxide solution, and the procedure were guided by ultrasound. Omeprazole (6 mg/kg) was administered during 7 days. Antimicrobial therapy was also instituted with oxytetracycline (20mg/kg), for 14 days, every 24 hours. Nineteen days after surgery the clinical signs of abomasal ulcers disappeared.

Conclusions: Although described as a difficult procedure, the infusion of magnesium hydroxide solution direct into the abomasum was possible following abomasopexy, and associated with omeprazole therapy, were effective in controlling the condition.

Internal Medicine

P03-003-145

Pathological and molecular confirmation of transplacental transmission of OvHV-2 in sheep-associated malignant catarrhal fever of cattle

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Objectives: Sheep-associated malignant catarrhal fever (SA-MCF) is an important infectious disease of ruminants worldwide that is caused by ovine herpesvirus 2 (OvHV-2). OvHV-2 is transmitted predominantly by contact between infected and susceptible hosts, while the documentation of vertical transmission is rare. This report presents the pathological and molecular findings associated with transplacental transmission of OvHV-2 in cattle

Materials and Methods: A 3-year-old Holstein-Gir cow, located at a farm in central west Brazil, was examined at the Universidade de Cuiabá. This farm contained 250 cattle and small flock of sheep. Clinically, there was corneal opacity, lachrymation, bilateral, mucopurulent nasal discharge, fever, apathy, with ulcerative lesions at the oral cavity. In addition, the cow was bearing a four-month-old foetus. The clinical condition of the cow deteriorated, resulting in spontaneously death. A routine necropsy was done soon after death. Tissue fragments from the brain, lungs, kidneys, liver, and myocardium of the cow and her fetus (myocardium, lungs, kidneys, and liver) were routinely processed for histopathological evaluation. Selected tissue sections of the cow (brain and kidney) and foetus (brain) were maintained at -80°C until used for molecular diagnostics. DNA extracted from the brain (foetus and pregnant cow) was used in PCR assays designed to amplify specific amplicons of the OvHV-2 tegument protein gene, the glycoprotein C gene of BoHV-1, BoHV-5, and the 16S rRNA gene of *Histophilus somni*. Positive and negative controls were included in all PCR assays. All PCR products were separated by electrophoresis in 2% agarose gels, stained with ethidium bromide, and examined under UV light. The amplified PCR products were then purified and submitted for direct sequencing using the forward and reverse primers. The partial nucleotide sequences obtained were initially compared by the BLAST program with similar sequences

deposited in GenBank. Sequence alignment and phylogenetic tree were then created.

Results: The principal gross findings of the cow included corneal oedema of the right ocular globe, fibrinopurulent bronchopneumonia, petechial haemorrhages at the mesentery, thoracic, and pleural surfaces, ulcerative stomatitis and rhinitis, pulmonary oedema, lymphadenopathy, and bilateral multifocal haemorrhagic nephritis. A normal looking 30 cm in length (Crown-Rump) foetus was found within the placenta. Histopathological evaluation of the tissues from the cow revealed lymphoplasmacytic, necrotizing vasculitis within the kidneys, carotid rete mirabile, brain, lymph nodes, and lungs with lymphoplasmacytic meningoencephalitis and nephritis. The PCR assay amplified the desired amplicons of the tegument protein gene of OvHV-2 from the brain and kidney of the cow, and the brain of the foetus. Direct sequencing was done with PCR products derived from the kidney of the cow and brain of her foetus. Initial BLAST analyses revealed that these sequences share 98 - 99% identity with similar sequences deposited in GenBank. The phylogenetic tree clustered the two strains of OvHV-2 with other OvHV-2 viral sequences identified in ruminants from Brazil and wild ruminants from Germany. Moreover, the generated sequence identity matrix and sequence alignment demonstrated that the nucleotide sequences of these two strains (cow and her foetus) are 100% identical. Additionally, the bovine herpesvirus type 1 (BoHV-1), BoHV-5, and H. somni PCR assays yielded negative results.

Conclusions: The clinical and pathological findings observed in this cow and her foetus are similar to those frequently observed in cattle with SA-MCF, and are the characteristic diagnostic features of this disease. Additionally, the amplification and sequencing of OvHV-2 from the brain of the cow and her foetus confirmed the likely participation of this pathogen in the development of the pathological lesions observed. These findings suggest the involvement of this pathogen in the lesions described and demonstrated the transplacental transmission of OvHV-2 in SA-MCF of cattle.

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Internal Medicine

P03-003-146

Lesions and Microbiological Findings Associated with *Mycoplasma bovis* -infection in Finland

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Objectives: *Mycoplasma bovis* was detected in Finland for the first time in 2012. By November 2015 *M. bovis* has been confirmed in 52 farms: 17 dairy herds and 35 meat producing farms. The aim of this study was to describe the lesions and microbiological findings in samples in which *M. bovis* was detected by culture.

Materials and Methods: Bovine carcasses or tissue samples from altogether 1509 cases, excluding abortion, were examined pathologically in the diagnostic laboratory service of the Finnish Food Safety Authority (Evira) from January 2012 to November 2015. The diagnostic laboratory findings were reviewed.

All samples were analysed grossly and specimens for histopathology, bacteriology and virology were taken and analysed with standard methods when appropriate. When pneumonia, arthritis or otitis media was diagnosed macroscopically, samples were examined also for *M. bovis* and ureaplasma.

Conventional aerobic, microaerophilic and anaerobic cultivation methods were used for bacterial isolation. *Histophilus somni* was identified from culture using PCR. Bovine respiratory syncytial (BRSV) virus and bovine coronavirus (BCV) were detected with PCR. *Mycoplasma* and ureaplasma were cultivated in specific culture medium (in CO₂) and confirmed with PCR. If a serum sample was available, it was tested for *M. bovis* antibodies with ELISA (BIO K 260, Bio-X Diagnostics, Rochefort, Belgium).

Results: Pneumonia was diagnosed in 26%, arthritis 4% and otitis media in 1% of the pathological samples. *M. bovis* was identified in 38 samples (3%) including 24 carcasses and 14 tissue samples. Most of the *M. bovis* positive cases were calves under three months old (35%), 24% were 3-6 months, 22% were 6-12, and 16% were over 12 months old.

The most common pathological finding connected to *M. bovis* was pneumonia (35/38). Six calves had pneumonia and otitis media and two calves had pneumonia and arthritis. In two cases only otitis media was diagnosed and in one case only arthritis in a cow. Pneumonia was classified as: Acute/subacute severe fibrinosuppurative (9/38), acute/subacute severe fibrinosuppurative with concurrent chronic lesions (11), local chronic suppurative bronchopneumonia (11), and cases with only fibrotic healing lesions (4).

Multifocal eosinophilic caseonecrotic lesion typical of *M. bovis* infection was seen in 63% of the histological sections (21 lung and one synovial sample). Necrotic foci were recorded in all classes of pneumonia cases.

Other pathogens were isolated in 25/38 (66%) of the *M. bovis* positive lung samples: *Pasteurella multocida* (14), *Histophilus somni* (12), *Ureaplasma diversum* (8), *Trueperella pyogenes* (6), *Fusobacterium* sp. (5), *Mannheimia haemolytica* (1) and *Bibersteinia trehalosi* (1). BRSV was detected in 4 cases and BCV in 3 samples.

Serum samples were available from 24 of the 38 *M. bovis* positive animals and in 33 % (8/24) *M. bovis* antibodies were detected with ELISA.

Conclusions: *M. bovis* was mostly associated with acute or chronic pneumonia in young animals and several other pathogens were often isolated from the same samples. Otitis media was more common than arthritis. Only one third of the serum samples tested for *M. bovis* antibodies were positive.

Lameness

P03-003-147

Low Body Condition Predisposes Dairy Cattle To Lameness

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Objectives: The extensive impacts of lameness on herd performance have been widely reported. Low body condition score (BCS) has been identified as a risk factor for lameness; cows with BCS ≤ 2 (0 to 5 scale) have been reported as being more likely to be treated for lameness in the following 2 or > 2 to 4 months. This longitudinal study aimed to investigate the hypothesis that cows in low BCS are at increased risk of first and repeated lameness events. Effects of other risk factors such as body weight (BW) were also investigated. This work has been published open access in the Journal of Dairy Science (Randall et al., 2015).

Materials and Methods: Records were obtained for the 8 year period 1st Sept 2003 to 31st Aug 2011, from a total of 724 cows, managed at the Scotland's Rural College's (SRUC) Crichton Royal research farm. Locomotion scores (1 to 5 scale) and BCS (0 to 5 scale; 0.25 increments) were recorded weekly by trained assessors. An automatic weighing system recorded BW after milking 3 times daily. Health, production, and management data were recorded in a database. Locomotion score was the outcome variable and categorised as not lame (LS 1 to 2), mildly lame (LS 3) or severely lame (LS 4 to 5). BCS, BW and milk yield data were lagged by 2 through to 16 weeks to explore their longitudinal association with lameness. Mixed effect hierarchical models were used to explore the relationship between explanatory variables and lameness outcomes. Modelling was conducted in 2 stages;

1. Multinomial model for repeated lameness events; were used to explore the relationship between explanatory variables and long term repeated mild and severe lameness events.
2. Discrete time survival models for first lameness events in a) heifers and b) 2nd lactation and greater cows; were used to investigate the relationship between BCS and lameness, without the confounding effect of a previous lame event causing BCS loss.

Results: Descriptive statistics; The data set included a total of 79,565 cow weeks at risk. The proportion of lame observations (mild and severe) in each lagged BCS category, from 3 through to 16 weeks, had a trend of decreasing percentage of lame events from BCS < 2 to BCS > 3 ; BCS 2.5 had the lowest percentage of lame observations.

Modelling;

1. Repeated lameness events model; lagged variables for BCS and BW were correlated from 2 through to 16 weeks. Cows with BCS < 2 three weeks prior to a lameness event were at significantly greater risk of lameness compared with other BCS categories. Risk decreased as BCS increased. Low BW cows (< 550 kg category) were at significantly increased risk of mild or severe repeated lameness events compared with cows in heavier BW categories (550 – 700 and > 700 kg).
2. First lifetime lameness event models; a) Heifers; BCS and BW were not associated with an increased risk of lameness. b) ≥ 2 nd lactation cows; cows with BCS ≥ 2.5 16 weeks prior to a first mild lameness event were at reduced risk of lameness compared with BCS < 2 . The same effect was seen at BCS = 2.25 8 weeks prior to a first severe lameness event.

Conclusions: These findings indicate that BCS < 2 is associated with the greatest risk of mild or severe lameness, and that risk decreases with increasing BCS. Results suggest that maintaining BCS ≥ 2.5 may be optimal for reducing the risk of lameness in dairy cows. These findings were relevant for long-term repeated lameness events and first time lameness events in second lactation and greater cows. Low BW (independent of BCS) was also identified as an important risk factor for lifetime risk of repeated lameness events. This study provides evidence to support targeting management towards maintaining BCS to reduce the risk of lameness in dairy herds.

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Lameness

P03-003-149

Cross sectional study on prevalence of lameness and foot disorders influencing welfare of Mediterranean buffaloes

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Objectives: Lameness represents the third most important health-related cause of economic loss in the dairy industry after fertility and mastitis. Although, dairy Mediterranean Buffaloes (MB) and dairy cows share similar breeding systems predisposing to similar herd problems, published studies exploring its relevance and role in these ruminants are still rare and incomplete. It was the aim of this study to assess prevalence of clinical lameness and foot disorders observed during routine foot trimming of a population of 1,297 MB housed in four different free-stalls.

Materials and Methods: Trimming procedures were performed once in all the multiparous MB (≥ 2 lactations), whether lame or not, present in the farms enrolled, between April 2014 and March 2015 in Latina District, Middle of Italy. Presence or absence of foot disorders (FDs) as well as their localization were recorded at claw trimming. Prevalence of the observed FDs, motility index (number of cows not lame/number of multiparous cows in the herd) and lameness index (number of cows lame/number of multiparous cows in herd) were also calculated. Locomotion score [LS; from 1 (non-lame) to 5 (severely lame)], body condition score [BCS; from 1 (emaciated) to 5 (obese)] and cleanliness score [CS; from 1 (no manure) to 4 (manure up to the leg)] were

evaluated of each MB affected by FD and used as welfare indicators. For descriptive statistical analyses, dedicated software (SPSS, Version 17.0, Chicago, IL) was used.

Results: The overall prevalence of animals affected by FDs was 17.65% (229/1,297), while motility and lameness index were 84.12% (1,091/1,297) and 15.88% (206/1,297), respectively. Claw overgrowth (OG) was present in 96.06% (220/229) of cases with FD, corkscrew claw (CC) in 89.52% (205/229), interdigital phlegmon (IP) in 5.24% (12/229), white line abscess (WLA) in 4.80% (11/229), digital dermatitis (DD) in 0.44% (1/229) and interdigital hyperplasia (IH) in 0.44% (1/229). Simultaneous presence of two disorders was recorded in 96.51% (221/229) of MB: OG + CC occurred together in 92.76% (205/221), OG + IP in 1.81% (4/221), DD + IH in 0.45% (1/221) and OG + WLA in 4.98% (11/221). No lameness caused by orthopedic pathology located outside the feet was detected during the investigation. Except from 23 MB with simultaneous occurrence of OG + CC, the presence of FDs was always associated with lameness (LS>2). Regarding the BCS, none of the MB with FDs were over-conditioned (BCS \geq 3.75), while 80.35% (184/229) were normal-conditioned (BCS \geq 2.75 and \leq 3.5) and 19.65% (45/229) were under-conditioned (BCS \leq 2.5). The CS was 4 in 50.22% (115/229) of MB affected with FDs, while it was 3 in 9.61% (22/229), 2 in 6.55% (15/229) and 1 in 33.62% (77/229).

Conclusions: During the current investigation, FDs still considered virtually absent in MB until now, were detected associated with clinical lameness and decrease of animal welfare. Although characterized by a lower prevalence than in dairy cows, disorders not only related to heritable factors but also to poor herd health management were observed for the first time also in these dairy ruminants

Lameness

P03-003-150

The Icar Claw Health Atlas – The Result Of An International Cooperation Of Claw Health Experts For Harmonization Of Terminology Of Bovine Claw Lesions

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Objectives: Documentation of claw health data during regular trimming visits has been identified as a valuable source of information on claw disorders on a cow and a herd level. However, heterogeneous documentation practices complicate their routine collection and use for genetic evaluation of claw health.

In 2014, the ICAR Working Group for Functional Traits (ICAR WGFT) started an initiative together with international experts with the aims to assess the situation of recording of claw health in the different member countries, and to establish an international harmonization of the definitions of claw disorders and a standardization of their terminology.

Materials and Methods: An online-survey was carried out in 2014 among the 53 ICAR member countries. Based on the results, a list of claw disorders/lesions that were regarded to be relevant for documentation of claw health in dairy cows and for genetic evaluations was elaborated following several meetings and online conferences. After the harmonized descriptions of claw lesions were agreed by ICAR WGFT, high-quality photos of each of the claw disorders/lesions were collected, and the most representative examples were selected by voting. The harmonized

definitions of claw lesions and the selected illustrations were the basis of the new ICAR Claw Health Atlas.

Results: Responses from 18 of 53 countries (34%) showed that around half of them have a single national key for recording claw and foot disorders. Information is collected in these countries on 6 to 20 different disorders, in many of them with severity grades. Professional hoof trimmers were found to be the main source of claw data, often using hand-held electronic recording devices and herd management software (Fig. 1; 11 countries). However, use of paper forms is still common, and it was assumed that many claw trimming events are not documented at all. No exact figures about the documented and centrally recorded claw health information relative to the percentage of dairy cows under regular milk recording are available. In countries with routine genetic evaluations for claw health, data from claw trimming are already stored in a central database and are also used for herd management.

For 16 claw disorders/lesions harmonized descriptions (Fig. 2, 3) were established and, illustrated by representative photographs, assembled to the first internationally agreed reference, the ICAR Claw Health Atlas. This Atlas of 16 claw disorders/lesions was published in the official ICAR language (English) and is now available for translation into other languages for distribution to professional trimmers and farmers worldwide. Information on translation and access to a high-quality print version are provided by ICAR. The English version is freely available online for download on the ICAR website: http://www.icar.org/Documents/ICAR_Claw_Health_Atlas.pdf

Conclusions: The international collaboration of the ICAR WGFT and international claw health experts provided for the first time a harmonization of terminology and definitions of claw disorders in cattle. One key aspect of successful initiatives to build routine genetic evaluations for claw health is the development of an infrastructure for electronic documentation of claw health data on a national basis. The other important key aspect providing the collection of high quality data is the use of a standardized terminology of claw lesions worldwide, which is now provided with the ICAR Claw Health Atlas as a guideline.

Lameness

P03-003-151

Comparison of the true lameness prevalence in dairy herds with its estimation by dairy farmers and herd health veterinarians

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Objectives: Lameness prevalence in dairy herds can be determined using lameness scoring charts. The use of these charts is semi-quantitative and helps users to identify lame cows (especially slightly lame cows). Unfortunately, dairy farmers and herd health veterinarians rarely spend time assessing lameness prevalence in Québec (Canada). Thus, it remains unclear if their estimation of lameness prevalence in herds is accurate or not. The objectives of this study were to quantify the lameness prevalence in dairy herds from a specific veterinary practice and to compare this prevalence to its estimated values from dairy farmers and herd health veterinarians.

Materials and Methods: A cross-sectional study was conducted on 20 commercial dairy herds that were clients of the bovine ambulatory clinic of the Faculté de médecine vétérinaire of the Université de Montréal

(Saint-Hyacinthe, QC, Canada). Herds were visited once by two trained observers (research veterinarians) to score lameness and to quantify the herd lameness prevalence. Lameness was score based on standard a 5-point scoring chart. Before lameness scoring was initiated at the farm, the farm's owner and herd health veterinarian had to independently estimate the lameness prevalence of the herd. Everybody was blinded to each other's result. True lameness prevalence was calculated. The difference between true lameness and its estimations from farmers and veterinarians were also calculated and compared with a t-test. Inter- and intra-observer agreements were calculated for both research veterinarians.

Results: The median true lameness prevalence was 25 % with a range from 11 to 65 %. On average, dairy farmers underestimated the lameness prevalence of their herd by 13 percentage points (ranged from underestimation of 45 % to overestimation of 10 %; $P < 0.01$). On average, herd health veterinarians estimated a similar prevalence in their herds compared with the true prevalence (ranged from underestimation of 20 % to overestimation of 20 %; $P = 0.68$). Inter- and intra-observer agreement (κ) from research veterinarians were 0.84 and 0.86, respectively.

Conclusions: Overall, these results suggest that dairy farmers generally underestimate the prevalence of lameness in their herd. They also suggest that herd health veterinarians can generally estimate well the lameness prevalence in herds. Finally, they suggest that trained observers performing lameness scoring can provide very similar results.

Lameness

P03-003-152

Distal sesamoid bursa in cattle: radiography, fluid taking, injection techniques and fluid analysis

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Objectives: A common area of lameness in cattle is the digital region, especially the distal sesamoid bone, distal sesamoid bursa (DSB) and distal interphalangeal joint. A few techniques for injection into the distal sesamoid bursa (navicular bursa) or fluid taking have been described in cattle. In this study different techniques for approaching to DSB were compared. In another study physical, biochemical and cellular evaluations of the synovial fluid of the DSB were compared in normal and abnormal claws in cattle.

Materials and Methods: Five different techniques for entering to DSB were compared in cattle including distal plantar approach parallel with the coronary band, proximal plantar approach, distal plantar approach parallel with the sole, abaxial approach, and distal interphalangeal joint injection. For the approaches radiography was also used. In 22 normal (11 rear limbs and 11 forelimbs) and 27 abnormal (16 rear limbs and 11 forelimbs) hooves of both sexes of Holstein cows, the synovial fluid were collected from the DSB. Fluid was taken from the sesamoid bursa by the technique of previous study. Clinical observation of the animals before slaughtering, grass evaluation and radiographic interpretation of the hooves were the basis for allocation to either the normal or abnormal group. Physical characteristics of fluid, total nucleated cell counts, differential cell counts, protein and glucose concentrations and the activities of some enzymes were measured by standard methods.

Results: Actually the placement of the needle through distal plantar

approach parallel with the sole was the best for fluid taking or injection. The results of fluid analysis of bursa showed the parameters of DSB fluid are the same as other synovial fluids in cattle. The results revealed that the enzyme activity of the abnormal synovial fluid from the DSB significantly increased. Also, there were no significant differences between the mean concentrations of total protein in the normal and abnormal synovial fluid specimens.

Conclusions: Placement of the needle through distal plantar approach parallel with the sole was the best for fluid taking or injection. Although there was no significant differences of some cellular and biochemical parameters of synovial fluid, the differences of these variables in normal and abnormal hooves may be useful for the diagnosis of healthy and abnormal synovial fluid.

Lameness

P03-003-153

Reducing lameness in organic dairy herds following farm individual intervention measures

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Objectives: Between 2007 and 2010 a nationwide interdisciplinary intervention study was carried out in 106 German organic dairy farms in order to develop preventive animal health management strategies for mastitis and metabolic disorders and to validate this concept (Brinkmann et al., WBC 2012). Lameness is a risk factor for metabolic disorders in dairy cows and affects animal welfare. Thus, the aim of the present study was also to reduce lameness prevalence by farm-individual intervention measures related to housing and management.

Materials and Methods: Average herd size was 57 cows (range 18-252) and average milk yield 6,053 kg per cow and year (range 3,424-8,917). The initial farm visit focused on the health situation (here: lameness assessment through locomotion scoring using a 3 point scale according to the Welfare Quality® (2009) Assessment protocol for cattle) and a range of potential risk factors including housing, herd management, feeding and forage production. Using this information, farm-individual evidence-based advice was provided by the project team and discussed with the farmers. Interdisciplinary intervention measures to improve udder and metabolic health were implemented in all farms and lameness was specifically addressed in 28 farms. The most frequently implemented measure was the introduction of a regular functional claw trimming or a shorter claw trimming interval (18 farms) followed by measures such as improvement of the cubicle base and of floor properties in the alleys (e.g. grip, deburring of edges, cleaning frequency) (10 farms). Effectiveness of intervention was monitored after one and two years, respectively (in total four farm visits within two years). Mixed models for repeated measures were used to analyse lameness prevalence at herd level.

Results: The average baseline lameness prevalence in all 106 project farms (visit 1) was 17.3 % and ranged from 0 to 63 %. Cubicle housed

herds tended to show more lameness (18.2 %, 88 farms) than cows kept in straw yard systems (12.6 %, 18 farms; $p=0.068$, Wilcoxon test). In the course of the observation period, lameness prevalence decreased to 13.2 % (visit 4; range 0 – 63 %). Considering the 28 farms which implemented measures specifically intended to improve the lameness situation, lameness prevalence significantly decreased from visit 1 to visit 4 (26.1 -> 15.9 %, $p<0.001$) whilst it did not change in the remaining 78 farms (control group) (13.4 -> 12.2 %, ns). Furthermore, intervention farms showed a significant reduction in prevalence of severely lame cows from visit 1 to visit 4 (9.3 -> 4.2 %, $p<0.001$) whereas there was again no significant change in control farms (4.6 -> 3.6 %, ns).

Conclusions: Our study showed that prevalence of lameness in organic dairy cows may be reduced by implementing farm-individual intervention measures. The results demonstrate the benefits of preventive herd health concepts: dairy farmers who implemented farm-individual intervention measures related to housing and management successfully reduced lameness in their herds. We therefore expect our findings to encourage the implementation of preventive herd health concepts in organic dairy farming.

Comments: The authors warmly thank the participating farmers for their interest and willingness to participate in the study and their generous hospitality. Furthermore the Federal Agency for Agriculture and Food (BLE) is gratefully acknowledged for financial support in the context of the Federal Organic Farming Scheme (BÖL), grant no. 07OE012-022 'Health and performance of dairy cows in organic farming'.

Lameness

P03-003-154

Identification of BDD-associated treponemes in Brazilian cattle

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Objectives: Bovine digital dermatitis (BDD) is an infectious foot disease that seriously compromises the welfare and productivity of affected cattle. Disease is currently assumed to be of multifactorial origin, with treponemes and possibly other, thus far unidentified infectious agents having a major role in BDD development and persistence. Here, BDD tissue obtained from 35 bovines in Paraná, Brazil, was assessed for the presence of treponemal DNA, and the most abundant Treponema types per lesion were identified.

Materials and Methods: Collected BDD tissue was subjected to DNA extraction using a commercial kit, followed by beta action PCR to confirm the PCR-compatibility of obtained DNA isolates. Then, the latter were PCR screened for the presence of treponemal DNA using a pair of pan-Treponema primers, i.e. 5'TT and 3'TT, as already published. Sterile water and Treponema-free bovine tissue DNA served as no template and negative controls. Amplification products were analysed by gel electrophoresis and visualized by ethidium bromide staining. Amplicons of sufficient quantity and anticipated size were gel-extracted and subjected to bi-directional sequencing.

Results: In total, 32 of 35 BDD DNA isolates scored positive by TT-PCR, presenting as weaker bands in 8, and as prominent bands in 24 cases. Gel extraction and subsequent bi-directional sequencing of the

latter resulted in the identification of the most abundant Treponema types present in the individual lesions, i.e. Treponema pedis in 9, T. phagedenis and T. medium in 5 cases each, feline oral treponemes in 4, T. refringens in 3, T. putidum in 2, and T. denticola and canine oral treponemes in one lesion each. A single Treponema type was detected in 15, two types in 9 of the 24 lesions.

Conclusions: As expected, the majority of BDD lesions tested positive for treponemal DNA. Overall, T. pedis, which is commonly found in BDD lesions in central Europe and the UK, was most frequently detected in Brazilian cattle. This observation further supports the concept of a causal association of T. pedis with BDD. In contrast, the Brazilian cows harboured typically BDD-associated treponemes such as T. medium, T. denticola, or T. phagedenis to a much lesser extent than European BDD-affected cattle, indicating that BDD-associated Treponema types vary geographically. However, more work and larger sample sizes are needed to confirm this theory.

Comments: Interestingly, four different lesions were shown to harbour feline oral Treponema types. In analogy, it could be shown previously that BDD lesions of Austrian cattle frequently contain canine oral treponemes. These findings emphasize the wide host range of Treponema sp. and support the concept of high cross-species infection rates.

Lameness

P03-003-155

A Randomized Controlled Trial Of 50 M2 Digital Dermatitis Lesions With A Non-Antibiotic Gel Containing Chelated Copper And Zinc

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Objectives: Digital Dermatitis (also known as Mortellaro's disease or hairy heel warts) is a chronic infection of the digital skin in cattle, causing lameness, economical loss and decreased welfare. In Europe, a non-antibiotic gel containing chelated copper and zinc has been registered for the treatment of Digital Dermatitis after clinical comparison with an antibiotic spray (Holzhauer et al., 2011). In Canada, no medication was officially registered for the treatment of Digital Dermatitis. To obtain such registration, a randomized controlled clinical trial was performed to investigate the efficacy of the non-antibiotic gel in Canadian dairy cows.

Materials and Methods: On three farms, a total of 50 active Digital Dermatitis M2 lesions were randomly divided in two groups: A) 25 lesions were treated with the non-antibiotic product (Intra Hoof-fit Gel); and B) 25 lesions were used as untreated controls.

Group A was treated with the non-antibiotic gel according to the following protocol:

Day 0: Approximately 5 grams of gel was applied on the lesion with a brush, then covered with cotton wool and maintained in place by an elastic bandage.

Day 3: The bandage was removed, and approximately 5 grams of gel was applied again with a brush, no bandage.

Day 7: Approximately 5 grams of gel was applied with a brush, no bandage.

The trial lasted for 10 days, and on day 0, 7, and 10, the animals were restrained in a hoof-trimming chute. The affected hoofs were cleaned with a towel, and lesion characteristics were scored. On day 0 and 10, a digital photograph of the lesions was taken.

In this study, "cure" was defined as the transition of the M2 type lesion to an M0, M1, M3 or M4 type lesion. All scoring and treatment of Digital Dermatitis was performed by the independent investigator (Emil Sabau). The results were statistically analyzed using a Fisher's exact test, $p < 0.05$ was considered significant.

Results: On day 7, each single lesion of the gel-treated group had already changed from a red and active M2 to the healing M3 phase covered with a scab (23 out of 25 lesions) and the non-painful chronic M4 phase (2 out of 25 lesions). This was in sharp contrast to the untreated lesions, that all remained red and active M2 lesions on day 7. On the final day, day 10, this striking difference remained unchanged. The association between groups and outcomes was statistically significant with a p value of < 0.000001 .

Conclusions: Registration of an antibiotic-free treatment for Digital Dermatitis in Canada required a clinical comparison with untreated lesions. All lesions treated with the non-antibiotic gel cured, while all untreated lesions remained active. This all-or-nothing effect confirmed the efficacy of the non-antibiotic gel as a powerful treatment for Digital Dermatitis in cattle.

Comments: Disclosure of interest: GL, RvB, HvdV and CV are employees of Intracare B.V., KV is the local distributor of Intracare hoof products in Canada.

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Lameness

P03-003-156

Claw Lesions During Early Lactation Are Not Preceded By Changes In Oxidative Status From Dry-Off To Early Lactation

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Objectives: Lameness is a major health disorder of dairy cattle and there is now growing evidence suggesting that lameness might be triggered during the transition period. Oxidative stress (OS) occurs during the transition period in dairy cattle and a recent study suggests that OS associated episodes of lameness. The question remains whether OS occurs due to lameness or if OS around the time of calving may precede the development of claw lesions. Therefore, this study investigated whether cows showing claw lesions during early lactation had a greater pro-oxidant and inflammatory status throughout the dry period or the start of the lactation.

Materials and Methods: For this cross-sectional study, blood samples were taken from 30 cows from the same herd at dry-off, movement to the close-up pen and between 3-7 DIM and the concentration of the acute phase proteins haptoglobin and serum amyloid A, reactive oxygen and nitrogen species (RONS) and antioxidant potential (AOP) were quantified in serum. Animals were followed up to 120 DIM

and grouped ex-post into one of the following health categories: (1) exclusively hoof lesions (HL); (2) other clinical or subclinical postpartum disease/s (OPD; such as retained placenta, metritis, mastitis, displaced abomasum, excessive lipid mobilization and/or hyperketonemia); or (3) non-diseased (ND). Three cows were culled before the completion of the study and hence their data were excluded. The HF category was finally composed by 7 cows, OPD by 12 and ND by 8 cows. Mixed models with repeated measures built on JMP Pro v11 were used to investigate the effects of time, health category and their interaction on the concentration of markers of oxidative status (RONS and AOP) and acute phase proteins.

Results: We hypothesized that those animals suffering from hoof lesions would show a higher pro-oxidant status than healthy animals during the dry period and the onset of lactation. However, contrary to our initial hypothesis, the health category did not show a significant effect on any of the oxidative status markers employed in this study. The previous studies that reported increased oxidant activity in cows with claw lesions or altered gait measured oxidative status biomarkers when the cows were already showing clinical signs. Therefore, the greater pro-oxidant state found in this studies could just have developed shortly before the onset of clinical signs of lameness, or be a consequence of the inflammation of the injured tissue.

Conclusions: The development of claw lesions during early lactation has not been associated either with a greater pro-oxidant or inflammatory status during the dry period or the start of the lactation, thereby suggesting that these changes, reported in other studies, either take place closer to the onset of clinical signs of lameness or develop as a consequence of the inflammation of the injured tissue.

Lameness

P03-003-157

New ways in prevention of digital dermatitis using an organic acid based germicide

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Objectives: Footbaths containing carcinogenic formalin or heavy metals such as copper sulfate are a commonly used tool to control prevalence of digital dermatitis in modern dairy farms. But these agents represent a serious hazard to human health and the environment. The aim of this study was to evaluate the efficacy of an alternative, organic acid based disinfectant which is less harmful, applied on every foot individually.

Materials and Methods: A total of 218 cows in two commercial dairy farms were included in the study. All animals underwent hoof trimming during the first visit (day -14). 182 feet presented with acute (M2) or chronic (M4 or M4.1) digital dermatitis lesions and were treated with salicylic acid. Success of treatment was documented. The disinfection regimen was started 14 days later (day 0): Three times a week hind feet were cleaned with a water hose in the milking parlor. After that the disinfection solution (EasyStride, DeLaval) containing lactic and formic acid, was sprayed on all right hind feet at a dilution of 4%. Left feet remained non-disinfected as a control group. Every foot was evaluated on day 0, 20, 49 and 79 following a modified DD lesion score (Doepfer 2009).

Results: On day -14 and day 0 there was no statistical difference in DD prevalence (acute DD lesions (M2): 98 feet (22%) and chronic DD lesions (M4 or M4.1) 84 feet (19%)), on day 49 ($p=0.007$) and day 79 ($p=0.01$) statistical analysis points out a significant difference between the two groups (sign test). The sprayed feet showed a significantly lower recurrence rate of clinical signs of DD than the ones that had only been washed. The pre-deluted disinfecting solution had a steady concentration of formic acid (0,45 %) and lactic acid (0,47 %).

Conclusions: In contrast to the use of footbaths where slurry contamination increases, individual application of a disinfectant after pre-washing is able to provide a steady concentration of the disinfection solution leading to better results in DD prevention. The organic acid based disinfectant meets the expectations of producers and consumers in a reliable and nonhazardous chemical. The topical spray application resulted in an acceptable prevention on new DD lesions. Additionally organic acids do not cause relevant residuals neither in milk nor in manure. It should be noted that hoof disinfection can only be part of a consequent hoof health management.

Lameness

P03-003-158

Beef cattle foot health at the point of slaughter in the UK

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Objectives: Lameness has received little attention in beef cattle in the UK, despite being recognised as of major welfare and economic importance in dairy cattle and sheep. Scarce research in this field means that even basic information, like prevalence and common types of lesions, is lacking.

This study aims to establish whether, and to what degree, foot lesions or abnormal conformation are present in prime beef cattle at the point of slaughter.

Materials and Methods: Over 120 lower cattle limbs were collected at one abattoir over several months. Cattle were prime beef animals under 30-months-of age. Limbs were selected randomly from the waste bin, therefore no back-tracing to animal ID, exact age or breed was possible. Using claw size and shape, feet were identified as front or back, and left or right.

For claw conformation, claw length was measured along the dorsal wall, sole surface assessed for evenness and alignment with the weight-bearing wall, and height difference between lateral and medial claw noted.

The interdigital space was examined for skin disruption, infection and hyperplasia. Extent and stage was noted for any digital dermatitis lesions. Horn was removed superficially from the sole using a foot trimming knife to allow visualisation of any lesions. The presence, extent and location of any sole haemorrhage, sole ulcer, white line lesion or heel horn erosion was noted.

Results: The majority of feet displayed at least one lesion, based on preliminary analysis. Heel horn erosion and sole haemorrhage were common, with each present in about two-thirds of limbs. Interdigital hyperplasia, in contrast, was rarely present. Severity of lesions ranged from mild to severe, like a deep toe lesion or interdigital necrobacillosis.

Claw length varied from seven to ten centimetres. Occasionally, double or underrun sole horn was observed, and in about one fifth of claws the weight-bearing wall was higher than the sole surface.

Lesions were classed severe enough that the live animal would very probably have been lame in about one in five limbs (21%).

Conclusions: Feet of prime slaughter cattle displayed a high prevalence of abnormalities. Both abnormal conformation and foot lesions were found. The welfare regulations for cattle during transport and at slaughter were not fulfilled in about one-fifth of animals, in which lesions were regarded as severe enough to have resulted in lameness in the live animal.

These results suggest that foot abnormalities are present in cattle reared for meat to such a degree as to necessitate further studies.

Lameness

P03-003-159

Assessment of the footbaths contamination by dairy cattle manures under fields conditions.

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Objectives: Infectious diseases, such as bovine digital dermatitis, became one the most important causes of lameness in dairy cattle. Among medical actions, footbaths represent a useful alternative to treat concomitantly an important numbers of animals for a given period. The active substances used in footbaths can be challenged against different amounts of organic matter (OM), temperatures and/or pH changes, which could alter their bactericidal activity. The aim of our experiment was to determine under field conditions the variations of the temperatures, pH and OM amounts in footbaths challenged against different numbers of cows which walk through them.

Materials and Methods: The experiment was carried in 5 dairy cattle farms from western France. The herds had to be (i) in zero grazing system to avoid natural cleaning of the feet on pasture, (ii) be housed in cubicles where the cleanliness of the feet is expected to be worse than in strawyard housing system, and finally, (iii) the herd size was limited to a minimum of 100 cows milked twice a day, to homogenize the sampling period to 24 hours.

The hygienic status of the farms was determined by the overall feet hygienic score of the farm based on a specific grid focusing on the feet. A footbath filled with 250 liters of water was placed at the parlour exit for 24 hours. The temperature was continuously monitored through a capture device located in the footbath and in the barn (to monitor ambient temperature). After an homogenization of the footbath content with a shovel, 3 samples (1L/sample) were taken throughout the footbath at different places after the cow passages (0 to 200), to analyze the evolution of OM content and pH. Additionally, the number of cows that defecated into the footbaths was recorded.

Results: Preliminary results indicate that the OM content rise together with the increased number of animals that walk through, and mostly, by the number of defecations into the footbath, varying from 0.086 g/L before the first passage up to 40.3 g/L after the last measure. The temperature measured in the footbath varies across the day from 12.5 °C to 18 °C with less than 2% of variation in relation to the ambient temperature. Finally, the pH of the footbath varied within the 24 hours period from 6.70 to

7.76. After the first defecation the pH increased from 6.70 to 7.48, and then the measures varied in-between 7.32 to 7.76. The mean number of animals that had defecated into the footbaths rose to 10% after 200 cow passages. We are currently performing the data analysis in order to provide practical advices such as renewal frequencies or concentration of the product according to the hygienic status of the farm.

Conclusions: The results of the present finding support the need for further studies to test the effectiveness of disinfectant in presence of different OM content or pH conditions to adapt, if necessary, the concentration of the product in the footbath or the frequency of renewal.

Lameness

P03-003-160

Inter-observer agreement between foot trimmers on foot lesions in cattle

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Objectives: The majority of lameness cases are caused by foot lesions and it has been suggested to target lesion-specific causes of lameness in a herd, rather than making generalised preventative changes. Drawing conclusions about the epidemiology and risk factors for specific lesions from existing studies is problematic. In order to research the epidemiology and risk factors for specific causes of lameness further, a standardised protocol for categorising foot lesions is needed. The aim of this study was to evaluate the inter-observer agreement between 5 foot trimmers from one vet practice when recording foot lesions in cattle.

Materials and Methods: A variety of photographs of foot lesions was obtained from several archives belong to the authors and in total included 13 different lesions with 6 pictures of each type. 5 hoof trimmers working for the veterinary practice with varying years of experience were gathered together and shown each picture for up to 1 minute and asked to write down the name (colloquial terms were accepted) of the main lesion they observed. The results of each observer were compared with a 'gold standard' diagnosis which was made by an experienced veterinary practitioner. The percentage agreement (sensitivity), specificity and kappa statistic were calculated for each lesion and the strength of agreement was determined.

Results: The kappa value is a means of measuring the agreement between individuals with a maximum score of 1 indicating perfect agreement. On the basis of the kappa values, sensitivity and specificity the strength of agreement for each lesion was classified excellent, substantial or poor. Results indicated that the trimmers had good agreement (excellent or substantial) for the identification of solar ulceration, white line disease, toe necrosis, sole haemorrhage and horizontal and vertical wall fissures but poor agreement for digital dermatitis, interdigital necrobacillosis, heel horn erosion, interdigital hyperplasia, sole penetration, sole separation and 'bulb infection'.

Conclusions: Finding poor observer agreements is useful in alerting us to the need to improve training for foot trimmers or redefine the categorisation of these lesions. The variable results between lesions emphasises the importance of recording inter-observer agreement when carrying out studies focussing on specific foot lesions. The specificity of the lesion scoring was high, suggesting when a lesion is identified, it is identified correctly. The low sensitivity for some lesions suggests that

some lesions may be missed by the foot trimmer but may also reflect the limitations of assessing a lesion by looking at a picture.

Lameness

P03-003-161

High within- and between-observer agreement can be achieved in different professional groups applying a video mobility scoring system of dairy cows

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Objectives: Mobility scoring is widely used to monitor lameness prevalence in dairy cows. To ensure data validity, high within- and between-observer agreement is necessary. A video-based mobility scoring system offers the opportunity to assess groups of observers under standardized conditions. The objective of our study was to investigate within- and between-observer agreement of farmers, bovine practitioners, veterinary students, researchers and a cattle-inexperienced food sensory panel. Further objectives were to investigate the effect of educational background and preferences when assessing gait attributes.

Materials and Methods: An online questionnaire with video samples was developed based on recordings of lactating dairy cows walking on slatted floor. The König-Garcia mobility score (ranging from score 1 to 5) was developed following guidelines from sensory science (Garcia et al., 2015). This score evaluates cows during walking and does not require a cow standing. Video sequences were obtained in four herds and the sequences shortened and allocated into two tests, test A and test B. Each test consisted of 11 unique video sequences (11 cows) shown in replicates at random order. The authors assigned a reference mobility score to each video sequence. The different professional groups were tested at seven independent sessions with a total of 102 observers. The sessions were organized in: Introduction to test and König-Garcia mobility score, test A, short training session with cases, break, test B, final questions on preferred gait attributes. The data was analyzed using kappa coefficients and logistic regression models

Results: Crude weighted kappa for within-observer agreement in test A and test B ranged from 0.76 to 0.80 and 0.70 to 0.75. Combining both tests, sensory assessors had the lowest probability of perfect agreement with 55%, while fourth-year veterinary students had the highest with 72%, when adjusted for fixed effects of video sample and preferred attribute. In general, the differences between groups were low and even inexperienced observers achieved perfect within-observer agreement of 60%. Observers preferring the attributes arched back or the overall mobility score achieved highest perfect agreement.

The between-observer agreement was assessed by weighted Kappa and ranged from 0.67 to 0.72 in test A and 0.58 to 0.69 in test B. A higher level of between- and within-observer agreement could not be achieved after a training session. This could either indicate that the short training session was insufficient and further efforts are necessary to develop a training tool, which is able to increase both within- and between-observer agreement, or that the training in the middle confused the participants.

Conclusions: In conclusion, the mobility score achieved high within- and between-observer agreement, when compared with the available

literature, in all professional groups. The short introduction appears to be sufficient for even inexperienced observers to achieve high observer agreement. Consequently, the mobility score seems feasible for on-farm application.

References: Garcia, E., König, K., Allesen-Holm, B.H., Klaas, I.C., Amigo, J.M., Bro, R., Enevoldsen, C. (2015). Experienced and inexperienced observers achieved relatively high within-observer agreement on video mobility scoring of dairy cows. *Journal of Dairy Science* 98:4560-4571.

Lameness

P03-003-162

Use Of Extended Characteristics Of Locomotor Behavior For Automated Identification Of Lamé Dairy Cows

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Objectives: Lameness prevalence in dairy cattle and its impact on animal welfare is high. Thus, systems for automated lameness detection are increasingly important. This study was carried out firstly to detect differences in locomotor and feeding behavior between lame (group L) and non-lame (group C) cows and secondly to create an automatic lameness detection model, using data of the three-dimensional acceleration of the hind limbs and activity of the jaw.

Materials and Methods: Twelve non-lame and 41 lame multiparous German Holstein cows, housed in a commercial loose stall in Germany, were included in the study. They were equipped with two three dimensional accelerometers (RumiWatch®), attached to each hind limb, and one halter with noseband sensor (RumiWatch®) attached to the head. Accelerometers and noseband sensors had previously been validated. The devices recorded data of 3 consecutive days (recording period). Using video recordings, each cow's gait was scored on a 5-point scale before and after the recording period, and the mean value of 3 independent experienced observers was taken as definite gait score. Group C cows were defined as showing a gait score ≤ 2, group L as showing a gait score ≥ 2.5.

For comparison between group L and group C, the T-test, the Aspin-Welch test and the Wilcoxon test and for model creation, logistic regression and receiver operating characteristics (ROC) were used.

Results: Group L had compared to group C significant lower eating and ruminating time, fewer eating chews, ruminating chews and ruminating boluses, longer lying time and lying bout duration, lower standing time, fewer standing and walking bouts, fewer, slower and shorter strides and a lower walking speed (P < 0.0001 – 0.04), whereas ruminating chews per minute and ruminating chews per bolus, as well as walking time and number of lying bouts did not differ between group C and group L (P > 0.09 – 0.8). Using only accelerometer variables, we could create a model with a sensitivity and a specificity of > 90%. Additional use of noseband sensor data did not substantially improve the model.

Conclusions: The results of this study show that the newly developed RumiWatch® accelerometers are able to detect differences in a broad set of behavioral parameters in lame and non-lame cows. Models

respecting two accelerometers variables only, automatically identified lame cows with high accuracy. Additional use of the noseband sensor does not provide substantial improvement in accuracy. Management factors influence the behavior of dairy cows. Thus, a multicenter study is needed, taking into consideration various management conditions.

Lameness

P03-003-163

Plasma Metabolic Profiling Analysis of Dairy Cows with Laminitis based on GC-MS

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Objectives: Laminitis is a commonly occurring disease which could cause lameness, threatening dairy cows' welfare and causing the huge economic loss in dairy industry. In order to analyze the metabolic change in dairy cows with laminitis, metabolomics strategy was adopted in this study.

Materials and Methods: 20 plasma samples were collected from cows in the laminitis and healthy groups. Plasma metabolic profiles were obtained by utilizing the gas chromatograph-mass spectrometer (GC-MS) technology. Multivariate statistics including principal component analysis (PCA) and supervised partial least squares-discriminant analysis (PLS-DA) coupled with statistic analysis were conducted to compare the metabolite profiles between the two groups and identify the changed metabolites. Bioinformatics were analyzed to compare the metabolic difference and screen the potential biomarkers in laminitis.

Results: 37 metabolites were identified by GC-MS approach, in which 3 were up-regulated and 34 were down-regulated. By using multivariate statistics analysis and pathways enrichment analysis, we found diverse compounds with very significantly different (p < 0.01) pathways including fatty acid biosynthesis, Gly, Ser, Thr metabolism and biosynthesis of unsaturated fatty acids as well as significant differences in the pathways involved purine metabolism, methane metabolism, cyanoamino acid metabolism and phenylalanine metabolism and so on.

Conclusions: Lipid metabolism pathways and Gly, Ser, Thr metabolism pathway play important roles in occurrence and development of laminitis in dairy cows. Our findings reveal the metabolic disorders of laminitis in dairy cattle and provide further knowledge of mechanism and pathogenesis of laminitis.

Lameness

P03-003-164

METABOLIC AND MINERAL PROFILE IN THE PERIPARTUM: CLAW QUALITY AND CONFORMATION

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Objectives: The objectives of this study were to evaluate the variations in conformation measures, endurance, growth and wear of the hooves of dairy cows among the 30 pre-delivery days and 90 days of lactation, as well as changes in the metabolic and mineral profile of dairy cows postpartum period and variations in the occurrence of foot lesions of dairy cows among the 30 pre-delivery days and 90 days of lactation.

Materials and Methods: The research took place at a dairy farm located in Minas Gerais State with a herd with 120 lactating cows, producing 2,000 liters of milk/day. Was used 30 crossbred dairy cows (3/4 Holstein x 1/4 Gyr), between second and fourth calving, assessed between February and August 2012, in which were measured claw conformation, claw hardness, claw growth and wear and the occurrence of foot lesions at 30 days antepartum, at delivery, and at 30, 60 and 90 days of lactation. We took blood samples by puncture of the coccygeal vein weekly during the prepartum beginning in the fourth week before calving expected date, one sampling 24 hours after calving, and in the second, fifth, 10th, 15th, 21th and 30th days postpartum. Thereafter, we conducted analyses of triglycerides, cholesterol, glucose, gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) using the technique of spectrophotometry in automatic device and commercial kits. We used cutoff points of 1.2 mmol/L of BHB for subclinical cetose diagnose and below of 8.5mg/dl in postpartum for subclinical hipocalcemia. Was evaluated the changes in the energetic and mineral profiles at 5 and 10 days postpartum and their correlation with claw conformation, claw hardness, claw growth and wear and the occurrence of foot lesions.

Results: Was observed increase in the claw angle and heel depth and decrease of dorsal wall length between the 30 days' pre-partum and 90 days of lactation. The hardness of the claw decreased at day 30 of lactation in the measurements done on the wall and the sole-wall. In the white line and sole the hardness decreased at birth and at 30 days of lactation. The claw growth rate reduced and claw wear increased in the first month of lactation. Were identified correlations between the concentrations of calcium, phosphorus, magnesium and non-esterified fatty acids (NEFA) at postpartum and claw measurements evaluated in the first three months of lactation. Low concentrations of serum calcium were correlated with lower claw growth rate and the lower claw hardness. Low magnesium concentrations were correlated with higher claw wear rate. High NEFA concentrations presented correlation with lower claw hardness. The frequencies of digital dermatitis were 60%, 63%, 73%, 53% and 46%, respectively at 30 days antepartum, at delivery, and at 30, 60 and 90 days of lactation. The frequencies of interdigital dermatitis were 46%, 36%, 53%, 46% and 46%, respectively at 30 days antepartum, at delivery, and at 30, 60 and 90 days of lactation. The frequencies of bleeding soles were 13%, 16%, 26%, 40% and 60%, respectively at 30 days antepartum, at delivery, and at 30, 60 and 90 days of lactation. And the frequencies of white line disease were 10%, 33%, 33%, 36% and 46%, respectively at 30 days antepartum, at delivery, and at 30, 60 and 90 days of lactation.

Conclusions: Claw conformation, hardness, growth and wear and the frequencies of claw lesions were influenced by mineral and metabolic alterations in the transition period. Subclinical hipocalcemia and cetose can alter the health of hoof in cows during the early lactation.

Lameness

P03-003-165

The effects of lameness on reproductive parameters and milk production in dairy cows

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Objectives: The main objective of this study was to estimate the effect of lameness on some reproductive parameters and milk production in dairy cows using monthly locomotion scores as an indicator for lameness. Optimum reproductive performance is crucial for milk production. Lameness has the potential to affect fertility at all stages of reproduction. Explanations include changes in oestrus behaviour due to lameness (e.g. less willing to be mounted due to pain related stress and uneven bearing of weight) and sore limbs and hooves. Other explanations could include diminished appetite, reduced conception rates and weight loss.

Materials and Methods: Farms Services at Massey University, New Zealand, supplied the data for this study. During on farm herd tests, data were collected, including monthly locomotion scores 0 - 3 scale (0 and 1 non lame, 2 and 3 lame) and mating records from two dairy farms over 24 months. The management systems of the two farms were a spring calving system with 450 cows, and a split calving system with 230 cows.

Data were analysed using Statistical Analysis Software (SAS) version 9.3. The herd data was segregated into three categories based on age: young cows of 2 years of age (referred to as heifers), 3/4 year olds, and cows older than 4 years.

The effects of lameness on various reproductive parameters and milk production were analysed using regression analysis and analysis of variance (ANOVA). For the regression analysis, lame cows were scored as one and non-lame scored as zero. Fixed factors included age, farm and month of mating. The effect of individual cows was included as a random factor. Modelling was carried out using the GLIMMIX procedure. The effects of lameness on reproductive parameters, before and following mating, were tested using analysis of variance in the MIXED procedure. The analysis of variance was also used to estimate the differences between: milk yield, days open, days to first service, number of services per conception and inter-calving interval among cows for each age category.

[LR1]0-3 scale (0 and one not lame, 2 and 3 lame)

Results: The effect of lameness on days open, number of services and days to first mating was inconsistent. The difference in days open of 92 days between lame and non-lame cows in 'before mating 2011', and a difference of 19 days between lame and non-lame cows 'following mating 2011' ($p < 0.05$). The difference of 20 days 'before mating 2012' and 8 days 'following mating 2012' for lame and non-lame cows ($p < 0.05$). A difference of 0.2 services was found between lame and non-lame cows 'before mating 2012', and a difference of 1.3 services between these cows 'following mating 2012' ($p < 0.05$). Regarding days to first service, a difference of 11 days 'before mating 2012' and 3 days 'following mating 2012' was found between lame and non-lame cows ($p < 0.05$).

All age categories displayed a 'normal' left skewed lactation curve with an increase in early lactation until 50 days, followed by gradual decline thereafter. The heifers consistently had the lowest average 305-day milk production (4705.4 ± 25.36 and 4941.4 ± 64.19 L) followed by cows aged 3/4 years old (5396.1 ± 24.17 and 5934.7 ± 41.80 L). Highest milk yields were recorded for older than 4 years cows (5986.7 ± 24.85 and 6447.9 ± 38.44 L for split and spring calving groups).

In 2011, heifers produced a greater total milk yield when lame (5058.2 ± 40.89 and 5022.0 ± 23.27 in lame and non-lame group respectively).

In contrast, in 2012, heifers produced a greater milk yield when non-lame (4975.4± 13.78 and 5006.9± 12.73 in lame and non-lame group respectively). Cows of 3/4 years produced more milk when non-lame.

Conclusions: This study could estimate the impact of lameness on milk production and fertility but one of the key costs of lameness (increased risk of culling due to reasons other than fertility) could not be measured. Results of this preliminary study were inconclusive, thus lameness may or may not have a significant effect on reproductive parameters and milk production. Overall, this study did show that lameness causes significant negative effects on the number of inseminations required for a cow to conceive and days open.

Lameness

P03-003-166

The risks and economic impacts of Traumatic Recumbency in 233 UK Dairy Herds

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Objectives: A series on farm meetings were conducted on 26 farms in the UK focusing on reduction of emergency culling as part of a carbon reduction project funded by Arla Foods and facilitated by Kite Consulting. The farmers highlighted traumatic recumbency as a major source of economic loss with a highly variable incidence between farms. The objective was to highlight the incidence and major contributing reasons of traumatic recumbency.

Materials and Methods: The survey was a non-random survey of the participating herds. Attendance to the meeting was voluntary and herds with a higher incidence of emergency culling may have attended. Data was gathered from 234 herds (44,451 cows, average herd size 192) using a questionnaire during the meeting. The data was based on the farmers recollection on "slip" rate (the estimated proportion of cows where their body contacted the ground every week) and the estimated proportion of the cows culled due to traumatic recumbency per year ("splints"), stuck in cubicles and other traumatic reasons. The farmers were also asked to identify the high-risk areas and cow risk factors for traumatic recumbency. The cumulative results were discussed with the farmers as the programme progressed. Due to the traumatic nature of the events the farmers were able to readily recollect the estimated number.

Results: Median culling rates for traumatic recumbency were 1.0% (range 0-6.3% Q1, 0.6%, Q3 1.9%). The median slip rate (% herd falling where body or udder touches the ground per year) was 52% (ranges 0-543%, Q1, 23% Q3, 93%). The top 3 cow risk reasons for recumbency were freshly calved, oestrus behaviour and calving problems. The top 3 risk areas were un-grooved concrete, congestion points and sloping areas. The farmers were broadly unaware of the variation between farms and accepted their own incidence as normal. The vet was seldom involved in discussions on how to reduce the incidence. The economic impact was discussed and this prompted the farmers to take action to reduce the incidence using a variety of techniques. A number of the farmers discussed how heat expression was difficult and it is possible that the economic impact of the type floor surface may have additional impacts on fertility and udder health.

The estimated profit opportunity from reducing traumatic recumbency from bottom quartile to top quartile was 0.61 pence per litre equating to a

herd benefit of £9,081 for the average herd based on culling costs alone. This is likely to be an underestimate of the true cost.

Conclusions: Reducing traumatic recumbency should be prioritised within farmer extension programs and more research is required to identify the correct combination of grooving and flooring surfaces in UK dairy herds to optimise cow welfare. Farms with incidences of traumatic recumbency above 0.5% (1 in 200 cows) per year should be provided with practical advice on how to reduce the incidence. The collection and discussion of the farmers' data was a highly effective method of highlighting the profit opportunity of reducing traumatic emergency culls.

Lameness

P03-003-167

Impact of oral meloxicam administered alone or in combination with gabapentin on experimentally-induced lameness in beef calves

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Objectives: Lameness is one of the most important welfare challenges in livestock production and is considered a chronic pain syndrome because hyperalgesia persists for at least 28 d after the causal lesion has resolved. Gabapentin is a γ-aminobutyric acid (GABA) analogue used extensively for the management of chronic pain in humans. Meloxicam (MEL) is a NSAID that is approved as an adjunctive therapy of acute respiratory disease, diarrhea and acute mastitis. This study examined the pharmacokinetics and analgesic effect of oral meloxicam (MEL) administered alone or in combination with gabapentin (GABA) in an experimental bovine lameness model.

Materials and Methods: Eighteen male British × Continental beef calves aged 4 to 6 mo and weighing 297 to 392 kg were randomly assigned to receive either (1) 0.5 mg/kg lactose monohydrate placebo (PLBO) (n=6); (2) 0.5 mg/kg MEL (n=6); or (3) 0.5 mg/kg MEL combined with 15 mg/kg GABA (MEL-GABA) (n=6) q24h for 4 d. The first treatment was administered 4 h after a chemical synovitis/arthritis was induced with injection of 15 mg amphotericin B into the left hind lateral distal interphalangeal joint. Changes in activity were evaluated continuously with pedometers. Contact force, contact area, contact pressure, impulse and stride length were recorded once daily with a pressure mat and visual lameness scores were determined by a masked observer using a 5-point scale. Cortisol and drug concentrations were determined daily by immunoassay and HPLC-mass spectrometry respectively. Outcomes were compared statistically using a random effects-mixed model and ANCOVA.

Results: There was a positive association between lameness scores and serum cortisol concentrations (P = 0.02) and a negative association between lameness score and step count (P < 0.0001), total force (P = 0.001), force applied to the lateral claw (P = 0.02), contact pressure (P = 0.005) and impulse of the lateral claw (P = 0.01). Step count was greater in MEL calves compared with PLBO (P = 0.008) and MEL-GABA (P = 0.04) calves. Impulse was greater in the MEL-GABA calves compared

with the PLBO calves ($P = 0.03$). There was an inverse relationship between plasma MEL concentrations and lameness score ($P = 0.02$) and a positive association between MEL concentrations and force applied to the lateral claw ($P = 0.03$), total contact pressure ($P = 0.03$) and impulse on the lateral claw ($P = 0.02$). There was a tendency towards a positive association between GABA concentrations, total impulse and impulse on the lateral claw ($P = 0.08$) and a negative association between GABA concentrations and step count ($P = 0.08$).

Conclusions: The results of this study suggest that MEL administered alone or in combination with GABA reduced the severity of lameness in calves following induction of lameness with amphotericin B. These findings have implications for developing analgesic protocols in lame calves that address both production and welfare concerns.

Lameness

P03-003-168

Dyskeratosis and laminar distortions development assessed by sequential hoof biopsies in beef cattle subjected to a high-grain diet on feedlot

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Objectives: The objective was to describe histological characteristics and to measure the epidermal laminae length of the bovine laminar tissue assessed by sequential hoof biopsies before and after high-grain diet on feedlot.

Materials and Methods: 11 Nelore bulls (20 months old, mean weight of 295kg) were housed on feedlot (16 m²/head) for a period of 100 days. The diet contained grass silage (23%) and grain (77%) and was calculated in order to provide a daily weight gain of 1.3kg. Hoof biopsies were taken one week before and at the end of the feeding period with each bull restrained in stock and under perineural anesthesia of the distal left hind limb. After hoof scrubbing, a circular saw mounted in a rotary tool was used to create a rectangular defect (3 x 0.5 cm) parallel and 3 cm distal to the coronary band of the lateral claw. A specific tool named Falcão-Faleiros lamelotome was used to collect hoof the laminar biopsies. At the end of the feeding period, another fragment was taken at the same level from the new cornified tissue that grew from the coronary band pushing the hoof defect distad. Tissue samples were routinely processed for histology and stained with H&E and PAS.

Results: Laminar histological pattern was considered normal in all baseline samples. The mean (\pm SD) length of epidermal laminae was $1449 \pm 338\mu\text{m}$. In contrast, all biopsies taken at the end of the experiment were abnormal showing apoptosis and distortion. The length of epidermal laminae ($252.3 \pm 66.2\text{ mM}$) was reduced ($P < 0.001$) compared with baseline values. An abnormal dyskeratotic layer ($287.9 \pm 85.3\ \mu\text{m}$ of thickness) was observed abaxial to the stratum laminar in all samples. These findings are compatible with hoof capsule distortions commonly seen in cases of subclinical laminitis.

Conclusions: In conclusion, the use of sequential hoof biopsies brought new information about the development of hoof lesions compatible with subclinical laminitis in cattle subjected to a high-grain diet on feedlot.

Lameness

P03-003-169

A retrospective analysis of field data to investigate the prevalence of foot lesions in dairy cows in the South West of England. A comparison from 2008 to 2015

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Objectives: The recording of both incidence and prevalence of lameness of the bovine on a farm level is poor across the world with a limited ability to meaningfully collate data across multiple herds as well as relying on repeatable lameness nomenclature and recording system. This study retrospectively analyses the data from a large veterinary practice in the South West of England employing 7 trained veterinary-led foot trimmers. The study also compares the most recent lesion prevalence with similar analyses performed in both 2008/9 and 2012 to the results from 2014/5.

Materials and Methods: Seven qualified foot trimmers (certified to a Dutch Diploma or NPTC Level 3) were equipped with bespoke technology to record lesions from foot trimming sessions on farm at the zonal level on the cows foot. The trimmers met regularly to standardise both nomenclature as well as trimming technique. The records from all sessions were analysed to investigate the prevalence of the major foot lesions recorded by the foot trimmers. These results were then also compared with two similar prevalence summaries carried by the same authors in 2008/9 (UK Cattle Lameness Conference) and again in 2012 (International Cattle Conference, Bristol).

Results: For the 12 months to the end of October 2015, 1131 trimming sessions were carried out on 146 farms. 23,792 cows were presented for foot trimming of which 17,038 had all four feet examined. Lesions were recorded on 13,101 (55%).

Bruising of the sole was the most recorded 'lesion' on the foot. However, excluding bruising, the predominant lesions that are recognised to cause lameness in cattle were White Line disease (22%), Digital dermatitis (22%) and Sole Ulceration (15%). Claw horn lesions believed to be associated with treponemes such as Toe necrosis and other so called 'non healing' lesions accounted for 8% of the lesions. Following further training the recording of sole fracture has risen to 3% of all lesions. A comparison is also made to data from other areas in the world.

Conclusions: The three main lesions recorded by foot trimmers in this part of the South West of England remain White Line Disease, Digital Dermatitis and Sole Ulceration. The herds trimmed by the team tend to be those that regularly foot bath their animals and so as in previous reports it is worth noting that there may be a selection bias on this prevalence data which gives an apparent low prevalence of digital dermatitis. The study continues to demonstrate the difficulties in recording consistent, reliable quality data in the this field.

Lameness

P03-003-170

Impact of lameness on productive and reproductive performance of grazing dairy cows

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Objectives: The objective of this study was to access the impact of timing of lameness case occurrence on milk production and reproductive performance in grazing dairy cows. The objective of this study was to access the impact of timing of lameness case occurrence on milk production and reproductive performance in grazing dairy cows.

Materials and Methods: A five-year retrospective longitudinal study was conducted in one commercial dairy farm in Argentina (35°37' S, 61°22' W). Cow records (n= 11,061) from cows that calved and were dried off between January 2010 and December 2014 were retrospectively divided into four groups according to time in lactation when the first lameness case was reported: healthy cows (G0), first lameness case prior to first postpartum AI (G1), first lameness case between first postpartum AI and pregnancy diagnosis (G2), and first lameness case after diagnosed pregnant (G3). Non-lame (G0) and lame cows (G1-G3) did not have any other concurrent disease at the same time during lactation (uterine infections, anovulatory conditions, and mastitis). The lameness effect on milk production at 150 DIM (MP150), at 305 DIM (MP305), and on interval from calving to first AI (IC1AI) and from calving to conception (ICC) were evaluated. In addition, the lameness effect on the risk for being inseminated by 80 days in milk (DIM, [AI80]), pregnant by 100 DIM (PR100), and open by 200 DIM (OP200) were evaluated. The statistical models included the fixed effect of Group (0 through 3), year (2010-2014), season of parturition (spring, summer, fall, and winter), parity (1, 2, 3+), and their second-order interactions. Modeling was performed using a manual backward elimination method with an exclusion criteria set at P > 0.2. The GLIMMIX, PHREG and LIFETEST procedures of SAS were used.

Results: Milk production 150 and MP305 were 116 and 181 kg higher in G0 compared to G1 cows (5152±23 vs. 5036±43, P=0.023; 9876±53 vs. 9695±91; P=0.074), but there was no difference in MP150 and MP305 in G0 regarded to G2 and G3 cows. The G0 cows had 6 to 12.5 days shorter IC1AI compared to G1, G2 and G3 cows (67 vs. 79.5 vs. 73 vs. 75, P<0.001). The G0 cows had 30 to 62 days shorter ICC compared to G1, G2 and G3 cows (96 vs. 126 vs. 158 vs. 127, P<0.001). The risk for AI80 was affected by Group since the odds ratio (OR) were 0.34 (0.26-0.44) for G1, 0.59 (0.47-0.74) for G2, and 0.48 (0.40-0.57) for G3 compared to G0 cows (P<0.001). The risk for PR100 was also affected by Group since the odds ratio (OR) were 0.47 (0.40-0.61) for G1, 0.37 (0.30-0.46) for G2, and 0.68 (0.57-0.80) for G3 compared to G0 cows (P<0.001). The risk for OP200 was affected by Group since the odds ratio (OR) were 1.58 (1.23-2.04) for G1, 1.46 (1.18-1.81) for G2, and 0.95 (0.79-1.14) for G3 compared to G0 cows (P<0.001). Finally, the median days to conception were 96 for G0 cows, whereas they were 126, 158 and 128 for G1, G2 and G3 cows (P<0.001). Conversely, Group had no effect on the hazard ratios (HR) for pregnancy (P=0.801) given that the HR were 0.90 (0.66-1.22) for G1, 0.83 (0.67-1.03) for G2, and 0.93 (0.76-1.14) for G3 cows.

Conclusions: Lame cows have lower milk yield and take longer to get pregnant. In addition, the time at which the cow become lame is important given that its negative impact on both, milk production and reproductive performance, is greater when it occurs before conception in early lactation. In conclusion, cows diagnosed as lame in early lactation have worst productive and reproductive performance than non-lame herdmates under grazing conditions.

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Lameness

P03-003-171

Recently emerged clinical pattern of arthritis and mastitis associated with *Mycoplasma bovis* in Irish dairy cows

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Objectives: Respiratory disease is the most common clinical presentation of *Mycoplasma* (M) *bovis* infection in Irish cattle. Cases of arthritis have also occurred sporadically, predominantly in calves, young cattle or housed beef fattening cattle, often as a sequel to respiratory disease. Until 2013, arthritis or mastitis caused by M *bovis* were rarely diagnosed in Irish dairy cows. In 2013 and 2014, arthritis and/or mastitis cases, associated with M *bovis* in dairy cows occurred in 17 Irish herds. The objective was to describe the clinical pattern observed, diagnosis and control measures applied.

Materials and Methods: Samples of aseptically collected joint fluid or milk samples were subjected to microaerophilic culture on mycoplasma broth and agar plates and to polymerase chain reaction for M *bovis* (Sachse et al, 2010). Further information about the clinical presentation, number of cows similarly affected, time affected, their response to treatment and their outcome was sought by questionnaire survey and herd investigation visits by the authors. Also, the extent of mycoplasmal mastitis in the affected herds was determined by testing of milk samples from other cows. Cows detected with mastitis were segregated and milked last as part of a mastitis control programme.

Results: During the first four months of 2013 and 2014, a range of 2 to 28 dairy cows per herd presented with lameness, reduced flexibility and swelling of one or more joints, in 13 seasonal spring-calving, dairy herds. In each of these herds, cases of swollen joints occurred within three to seven weeks of the occurrence of its first clinical case. The severity of lameness varied. Most cases were refractory to treatment initially. The metacarpophalangeal joint (fetlock of forelimb) was the most frequently affected joint, but also the carpus, hock and metatarsophalangeal joint in other cases. M *bovis* arthritis was diagnosed by isolation of mycoplasma colonies and by polymerase chain reaction, from samples of joint fluid from one or more cows in 12 of those herds and from milk samples from cows in the 13th herd. Mycoplasmal arthritis occurred as a primary clinical presentation without any clinical signs of respiratory disease in the majority of the affected cows. Cases of M *bovis* mastitis were diagnosed, mostly in cows that were not affected by lameness, in 5 of those 13 herds. Mycoplasmal mastitis was diagnosed in cows in 4 further dairy herds for which no cases of arthritis were reported during 2013 and 2014. In some herds M *bovis* was also isolated from cases of arthritis or respiratory disease in calves. For each herd partaking in a mastitis control program, a cessation of occurrence of new cases of mycoplasmal mastitis occurred. Mycoplasmal mastitis was eliminated from herds, from which these cows were culled.

Conclusions: *Mycoplasma bovis* arthritis and mastitis were diagnosed in adult dairy cows in Ireland in a series of herd outbreaks affecting 17 dairy herds in the years 2013 and 2014. Control programs were applied to contain the extent of (and/or to eliminate) mycoplasmal mastitis from affected herds.

Acknowledgements: The contributions of staff of the Central Veterinary

Research Laboratory and Regional Veterinary Laboratories are appreciated. The pro-active involvement of veterinary practitioners and herd owners contributed greatly to this work.

References: Sachse, K., Salam, H.S., Diller, R., Schubert, E., Hoffmann, B., Hotzel, H. (2010), *The Veterinary Journal*, 186(3):299-303

Lameness

P03-003-172

Atlas Of Infectious Hoof Diseases For Practicing Veterinarians

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Objectives: Currently infectious hoof diseases are an emerging problem in Finland. Various diseases and their similarity in different stages of the disease, thus somewhat different treatment and prognosis can be puzzling. In some cases, incorrect diagnosis can lead to unnecessary antibiotic or other treatments. Therefore, to make a clinical diagnosis as correct as possible is crucial to practitioners, hoof trimmers and farmers.

Materials and Methods: During 2012 – 2015, a research project of infectious hoof diseases existed in Finland. Within this project 28 farms visits were made and 245 cows (287 hooves) inspected in case of infectious hoof diseases. Cows were included to the study based on suspicion of infectious hoof diseases. The main interest was interdigital phlegmon and whether the lesion was acute, healing or chronic. Additionally, digital dermatitis (DD), interdigital dermatitis (ID), heel horn erosion and interdigital hyperplasia were detected. All the inspected hooves were photographed for a more precise analysis of the disease. Four veterinarians, who were experienced in infectious hoof diseases, together made the final diagnosis of the hooves from the photos.

Results: From the various photographs a color atlas of infectious hoof diseases was established. Pictures of healthy hooves were also included. The atlas can be found for free in the webpages of Animal Health ETT <http://www.ett.fi/sisalto/sorkkaterveys>.

Altogether 287 feet were photographed. Interdigital phlegmon was detected in 133 feet of which acute 26.8% (77/287), healing 19.2% (55/287) and chronic 0.35% (1/287). ID and DD was detected less frequently 14.3% (41/287) and 18.5% (53/287) respectively, as well as interdigital hyperplasia 6.0% (17/287). On the contrary, heel horn erosion was detected in 88.5 % of the inspected feet (254/287). Only 4.5% (13/287) of selected hooves were considered healthy of infectious hoof diseases.

Conclusions: Difficulties in diagnosis making process were apparent. Occasionally, the anamnesis of the cow and the information about an antibiotic treatment were necessary to differentiate the healing process of an interdigital phlegmon from an acute lesion of DD. Altogether, many cows suffered from several infectious hoof diseases simultaneously.

Lameness

P03-003-173

Laminitis lesions in the hooves of the Goats picked up during hoof trimming in a herd of goats

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Objectives: objectives. The foot of a goat is one of nature's works of art; it is designed such that a goat can easily balance upon rocks and steep crevices with very little trouble. In Iran, goats are mainly raised on pastures and therefore they need foot inspection less frequently. Permanently confined management system requires regular foot trimming schedule once or twice a year depends on the herd conditions. In this survey, hoof morphometric was studied prior and after trimming in goats in different chronic laminitis conditions.

Materials and Methods: A flock of indigenous goats raised indoors were inspected for hoof problems. All the goats were inspected and trimmed if it was necessary. Care was taken not to overtrim or to induce an unwanted ulcer or so. 28 goats weighted 51.25 ± 6.22 kg with abnormal foot condition were trimmed. Fore limbs and hind limbs were trimmed. The dimensions of the hoof such as dorsal hoof wall length and heel height were measured before and after trimming. Any abnormal conditions such as ulcer, crack, over growth etc was recorded

Results: The goat hoof is solid and strong. The ideal cranial wall length after trimming was found to be 3.8 ± 0.4 cm, the heel height was best 2.0 ± 0.2 cm. During hoof trimming, deep grooves in the heel (heel horn erosion), hoof cracks, heel ulcer, toe ulcer, white line disease, curling toe over growth and medial claw heel over expansion (overgrowth) both in fore and hind leg were noticed and recorded. Medial claw heel overgrowth in fore and hind legs was found to be dominant lesions observed in this study (21.4 %).

Conclusions: Chronic laminitis like lesion found in this herd suggested that feeding management as well as heat stress and other stressful conditions could be the major risk factors. Excessive growth of the wall is common especially towards the toe, and excessive growth of the sole was seen, particularly at the heel. An average length of the toe and heel height was proposed as a guide line for hoof trimming routine process. A general rule about trimming goat's feet was that the hoof's hairline (the coronary line) should be almost parallel to the ground. Over-trimming was highly undesirable which cause permanent damage to the feet such as toe granulomas.

Lameness

P03-003-174

Epidemiologic survey of lameness and hoof lesions to establish a control program in Prata city.

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Objectives: Today hoof lesions and consequently lameness is considered one of the three most common occurrences in dairy cattle and despite the advancement of knowledge its prevalence is increasing over the years. To reduce this prevalence, socioeconomic works are needed. Therefore, this project was developed to determine the prevalence of lameness and hoof lesions and to assess the risk factors present in a representative area of Minas Gerais, Brazil. Furthermore, we want to know the understanding and perception of farmers about lameness for further development of a lameness control program.

Materials and Methods: We visited 48 dairy farms and examined 370 lactating dairy cows in the Prata municipality in Minas Gerais State, Brazil. All farms had a pasture-based system with concentrate supplementation and with average daily milk production/cow between 5 and 19L. In each visit 7 to 12 animals were randomly selected and had the four limbs evaluated. They were contained in a mobile trimming chute and hoof lesions were noted in a sheet. Lameness score of the whole herd were recorded using a scale of 0-3 according to Stokes et al. (2008). The risk factors and preventive actions presented in each farm were also noted using a model form. The animal use for the research was previously authorized by each farmer and this project was approved by the The Ethics Committee on Animal Experimentation of Universidade Federal de Minas Gerais.

During the visit, a semi-structural questionnaire was applied to the owner or farm's manager to know their perceptions about the hoof lesions in the farm. All the interviewees were informed about the purpose of the research and signing by free will a consent form.

Results: Lameness was a great problem in the region with 16.14% of animals being lame and 6.93% being severe lame. Farmers perceived only 64.71% of the lame cows, but, nonetheless, 34% of farmers claimed lameness to be the major problem they have.

Hoof lesions were presented in 97.2% of animals. The lesion with higher prevalence in decrease order was heel erosion (89.80%), white line disease (51.28%), digital dermatitis (DD) (31.12%), sole hemorrhage (26.8%), others (17.8%), overgrown claw (16.58%), parasitic coronitis by *Tunga* spp. (12.24%) and interdigital dermatitis (11.22%). We didn't find animals with sole ulcer.

Analyzing the whole scenario we concluded that the infectious lesions type was the most important, especially DD while laminitis has minor importance. The reasons why these diseases have become so important is the lack of information, biosecurity and preventive measures. The purchase of animals is a common practice and 85.11% of farmers said that they often buy animals without examining them for other diseases than for tuberculosis and brucellosis or take other preventive or biosecurity action. Only 6.25% of the farms used foot bath regularly. The way to treat the lesions was another important issue with 35.42% of farmers using systemic antibiotic and 27.08% saying that they used to do interventions but most often by a non-trained person.

The major obstacles reported by producers to reduce the lameness prevalence or to treat animals was lack of adequate facilities like a trimming chute, lack of trained professionals and lack of information.

Conclusions: Lameness is a great problem in the region and it is mainly caused by digital dermatitis. There are a large number of farmers that perceived lameness to be one of the greatest problems they face. They consider that the lack of proper facilities, lack of information and trained professionals are some of the causes. These factors are displayed in the absence of prophylactic and biosecurity actions as well as the improper treatment provided to animals.

To reduce lameness prevalence in the region is necessary to do a work with the objective to raise awareness and facilitate access to information of farmers and consultants that work in the region.

Lameness

P03-003-175

Hormonal, metabolic and behavioral stress response to treatment of claw horn lesions

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Objectives: The objective of this blinded, randomized and placebo-controlled study was to evaluate the hormonal, metabolic and behavioral stress response of dairy cows after pre-emptive administration of the nonsteroidal anti-inflammatory drug (NSAID) ketoprofen on the treatment of acute claw horn lesions.

Materials and Methods: Forty-two German Holstein dairy cows of one herd were studied; twenty-one cows (group L) with a lameness score of ≥ 2 (range 0 to 5, bi-weekly lameness scoring of herd) of one hind limb and a claw horn lesion (sole ulcer or white line disease) were enrolled and treated according to a pre-prepared randomization list either with ketoprofen or placebo administered 15 minutes before claw trimming in lateral recumbency (LR). Each lame cow was matched with a healthy control cow (control group; C) from the same herd according to parity and stage of lactation. Control cows were treated with placebo and also received claw trimming on a surgical tipping table. Claw trimming and treatment of claw lesions was performed according to the Dutch school (Toussaint Raven, 2003). The time span for claw trimming in controls was adjusted to claw trimming and treatment of lame partner cows. Blood samples were collected from the jugular vein before treatment, at the end of the period in LR and after 24 h by venipuncture. They were analyzed for cortisol, fatty acids, lactate and glucose. Fecal cortisol metabolites were measured before and 24 h after surgical intervention. Behavior on the surgery table was recorded as vocalization, movements of the treated leg and of the whole body, without differentiating the magnitude of movements. Results were statistically evaluated by ANOVA for repeated measurements and scores by means of Wilcoxon test (SAS package vers. 9.3).

Results: In lame cows mean lameness scores were by about 0.5 score points reduced compared to baseline scores ($p < 0.05$) immediately and also 24 h after surgical intervention. Immediately after surgical treatment concentrations of blood cortisol ($p < 0.05$), fatty acids ($p < 0.05$) and glucose ($p < 0.05$), as well as 24h after treatment of fecal cortisol metabolites ($p < 0.05$) were higher in lame cows than in control cows. Also leg movements during treatment occurred more frequently ($p < 0.05$) in lame cows compared to controls. Neither average vocalization scores during treatments on the surgery table nor strong whole body movements were different between groups. No group differences were found between lame and control cows 24h after treatment for blood levels of cortisol, fatty acids and glucose. Pre-emptive administration of ketoprofen revealed no significant effects on study parameter.

Conclusions: Treatment of claw horn lesions in acute stages as commonly performed by professional claw trimmers without anesthesia and analgesia appears to be painful for affected animals. Results provided no indications for a reduced stress response on claw treatment

by pre-emptive application of the NSAID ketoprofen. In view of animal welfare aspects, adequate pain management appears obligatory while performing any kind of claw treatment. However, more studies are necessary to elucidate the severity of pain associated with treatment of claw horn lesions and on the development of adequate and adjusted pain management protocols for such interventions.

Comments: Toussaint Raven, E. 2003. Cattle Footcare and claw trimming. The Crowood Press Ltd., Marlborough.

Lameness

P03-003-176

Treatment of long bone, metacarpal and metatarsal fractures in 202 calves and cattle

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Objectives: The description of this study is to compare the causes, location, configuration, treatment and outcome of conservatively and surgically treated long bone fractures in hospitalized cattle during a 10 year period.

Materials and Methods: The medical records and radiographs of all patients which were presented to the Clinic for Ruminants with Ambulatory and Herd Health Services, LMU Munich, between 2003 and 2013, for reasons of long bone fractures and fractures of metacarpal and metatarsal bones were retrospectively analyzed. Information extracted from the medical records included location and classification of the fracture, treatment (i.e. conservative vs. surgical), the documented clinical course, results of radiographic re-examinations, and the final outcome of therapy.

Results: A total of 202 cattle with 208 fractures were included in the study. The location of these fractures was as follows: 7 (3.4%) at the humerus, 12 (5.8%) at radius and/or ulna, 53 (25.5%) at the femur, 30 (14.4%) at the tibia, 61 (29.3%) at the metacarpus and 45 (21.6%) at the metatarsus. The majority (n = 134; 66%) of were less than two weeks of age. A total of 32 animals (16%) were presented between the age of 15 days and one year, while 36 patients (18%) were older than one year. Fractures of metacarpus and metatarsus were most often seen in calves younger than 2 weeks of age. The majority (57%) of the presented fractures were induced during assisted delivery. In 51 patients, no treatment trial was performed for reasons of poor prognosis or financial constraints. In the remaining 153 animals, 88 (57.5%) were treated conservatively and 65 (42.5%) surgically. In the subset of conservatively treated patients 70 out of 88 (79.5%) were treated successfully and showed a full recovery, whereas 33 (50.8%) of the surgically treated patients had a positive outcome. Fractures of the metacarpal or metatarsal bones could be treated conservatively with a cast or walking cast and confinement to a stable. Fractures of the long bones, such as humerus, femur, radius and ulna had to be treated surgically by internal fixation in most of the cases.

Conclusions: The surgical treatment was difficult, expensive and often had a cautious prognosis. Missed colostrums feeding and concurrent diseases like neonatal diarrhea, umbilical infections, bronchopneumonia and septicemia had a significant negative influence on fracture healing in newborn calves. Surgical fixation of newborn bones is more difficult than in older calves or in adult cattle because the structure of the long

bones is soft without a sufficient physical strength for implants. Presently osteosynthesis using locking compression plate technique proved more successful than the use of dynamic compression plates in calves.

Comments: The literature lies with the author.

Lameness

P03-003-177

A National Evaluation of Lameness in Canada: Farmers' estimates of lameness compared to in-stall lameness scoring and locomotion scoring

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Objectives: Lameness is a significant concern for farmers and consumers. Dairy Farmers of Canada (DFC) has established an Animal Care Assessment (ACA) through a ProAction initiative which assesses lameness using locomotion scoring (LS) and in-stall lameness scoring (SLS) techniques. Studies have explored farmers' estimates of lameness compared to LS, but there are no studies on farmers' estimates in Canada, especially when incorporating SLS. The purpose of this study was to determine the prevalence of lameness on Canadian dairy farms using the prescribed methods listed in the ACA, and to explore farmers' perceived lameness in their herds.

Materials and Methods: Data collected for this project was part of a larger and more comprehensive "National Dairy Study (NDS)." The NDS consisted of two phases. Phase I was comprised of a comprehensive questionnaire, and Phase II consisted of a follow-up farm visit.

Phase I was completed in Spring 2015. Every licenced dairy producer in Canada (just under 12,000) was invited to complete the questionnaire that addressed a range of health and management issues, including lameness. The questionnaire was available electronically, by paper copy, or by phone interview. The survey was reviewed by an advisory committee and piloted.

In Phase II, during the summer of 2015, farms that completed Phase I and agreed to participate in Phase II, were selected based on multiple criteria, including but not limited to provincial representation, participation in milk recording, as well as by convenience. For the lameness portion of the farm visit, which was part of a larger animal-based measures assessment, data were collected to gain insight on the prevalence of lameness. Prior to data collection, 16 farm assessors were trained to assess animal based measures such as hocks, BCS, cleanliness, and lameness. The training incorporated two lameness scoring techniques. The first was the traditional 5 point LS, with an overall assessment of limp (score 3, 4, or 5) or no limp (score 1 or 2). The second technique was the SLS system. The assessors had three days of training to insure repeatability and accuracy for all farms (n=378) assessed across Canada.

Results: Phase I was completed by 1,342 dairy producers (11% response rate). The regional distribution of the respondents was representative of the Canadian dairy farm population. 90% of respondents participated in milk recording. 60% of respondents had tie-stalls type barns, 38% free-stall barns, and 2% had pack barns.

In-stall lameness scoring revealed the mean percentage of lame cows on tie-stall farms (n=156) was 38%, with lameness ranging from 0% to 94%. Tie-stall farmer estimates for percentage of lame cows within

Phase I had a mean estimated prevalence of 8%, with estimates ranging from 5% to 34%.

Locomotion scoring identified that free-stall farms (n=222) had a mean percentage lameness of 26%, with a range of 0% to 100%. Estimates by free-stall farmers for percentage of lame cows within Phase I had a mean estimated prevalence of 8%, ranging between 5% and 23%.

These findings indicate that free-stall based farmers failed to recognize 18% of their lame cows (mild to severe limp), while tie-stall farmers failed to recognize 30% of their lame cows.

Conclusions: It is apparent, that worldwide, prevalence of lameness on farms continues to be underestimated by farmers, and Canada is no exception. It is also apparent that tie-stall farmers tend to have a higher prevalence of lameness within their herds, and tie-stall farmers tend to underestimate lameness even more when compared to free-stall farms

Lameness

P03-003-178

Case study about Radial nerve palsy during regular hoof trimming on dairy farms

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Objectives: On modern dairy farms regular hoof trimming is an indispensable requirement. Ever and anon cows suffer from radial nerve palsy after being put through a chute. Only poor literature is available relating to that specific topic. In this case study a multidisciplinary research founded the base for an adaptive treatment regimen in cattle.

Materials and Methods: 3 Simmental and 9 Holstein Friesian cattle, housed in free stall barns or tie stalls, showed weakness up to complete disability to bear weight on a front leg. Literature research revealed a lack of scientifically proven methods of appropriate treatment. By lifting the legs in the chute, muscles, nerves and joints are exposed to abnormal pressure. Both in tilt table chutes and in walk through chutes compression and extending forces can lead to structural damage in the front leg. The different external- and internal conditions (chute, time period, body condition, -size, age of the animal, housing, number of legs lifted at the same time) were compared to predict the most probable damage and establish an appropriate treatment.

Results: The radial nerve innervating among others the flexor muscles at the back of the upper arm and those at the front of the lower arm, winds around the proximal humerus right upon the bone. There is no padding underneath protecting the fragile nerve. Compression and extension during trimming can lead to nerve constricting edema of the surrounding tissue or partial disconnection of the nerve. Analgetic and steroids (e.g. Prednisolon) were injected to reduce pain, swelling and limit secondary damage. Frequent physio therapy helps to maintain muscle strength and showed a positive impact on recovery. This treatment protocol is based on the research experience shared with human-, horse and small animal surgeons. The cows were housed separately on a soft but shallow bedding to prevent decubitus ulceration and facilitate the forward moving of the affected, hanging down front leg. One cow needed a scotch cast bandage in addition to the treatment to support the leg which eventually resulted in complete recovery of the front limb too. Only 2 cows couldn't get back on their feet, due to poor housing conditions and severe additional health issues, and had to be put down. Depending on the continuity of animal supervision by the farmer, the quality of on

farm housing possibilities and the will of the animal to stand up, the cows went back to normal gait and could be reintegrated in the milking herd after an average of 7 days.

Conclusions: In case of a radial nerve palsy following the trimming in a chute, proper treatment and support can lead to full recovery. Excluding causes others than compression or extension of the nerve, dysfunction can be treated successfully. Hence, from an economical perspective the attempt of treating the animal is more reasonable than culling and replacement. Best prevention of radial nerve palsy is the knowledge of the anatomical structures on the front limb and its implementation into the routine of trimming.

Lameness

P03-003-179

Governmental control of lameness in dairy herds in Denmark

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Objectives: Lameness in dairy cows is a painful condition that decreases productivity and is a major welfare problem. Lameness is considered the primary cause for losses due to disease in Danish dairy production. Cows with disorders of the hooves and limbs will usually be subjected to considerable pain and suffering. According to the Danish Animal Welfare Act (DAWA), animals should be treated carefully and protected as well as possible from pain, suffering, fear, disablement and major inconvenience. The objective of the study was to investigate whether the dairy farmers were able to handle cows that were lame to severely lame in accordance with DAWA.

Materials and Methods: Thus DVFA has done a survey during the summer of 2015 in 100 larger Danish herds with lactating dairy cows. The observational study included one visit to every included herd by two trained official veterinarians between June and September 2015. At the visits all lactating cows were scored for lameness according to the Locomotion scoring of Dairy Cattle proposed by Sprecher which includes 5 grades for severity of lameness. The numbers of lame to severely lame cows were recorded and the handling of lame cows by the farmer and/or his veterinarian was assessed by the official veterinarians.

Results: In total 100 herds ranging from 80 to 450 dairy cows was visited. The number of lame to severe lame cows ranged from 0 to 9,6 %.

In 28% of the herds handling of lame cows were deemed incorrect or insufficient. In herds with lame cows that handled these correct the following factors were identified; placement of facilities for sick or lame cows close to milking parlours and/or automatic milking systems, dry og non-slibbery surfaces within the stables as well as close collaboration between farmworkers and the veterinarian on handling of lame cows.

Previous studies in Danish dairy herds have revealed a high percentage (10 – 12 %) of moderate to severely lame cows within the herds.

Conclusions: DVFA have the overall responsibility for carrying out on-farm controls of animal welfare provisions in Denmark. If non-compliances are found, the reaction may be either a warning, an enforcement notice or a report to the police. In this study 28 % of herds were given an enforcement notice and 1 herd were reported to the police for mishandling of lame/severe lame cows.

Studies as the one described enables veterinary authorities to follow occurrence of major welfare problems in modern dairy herds and demonstrate – if repeated – an approach to evaluate whether farmworkers handling of lame cows will improve over time.

Lameness

P03-003-238

Cortisol - Concentration before, during and after claw treatment in German Holstein cows – Testing of different substrates

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Objectives: Measurement of blood cortisol has been widely used as a method to assess the activity of the hypothalamic-pituitary-adrenocortical (HPA) axis in cattle. As non-invasive alternative to blood, cortisol was also measured in milk, saliva, lacrima and their metabolites in faeces.

The objective of the present study was to examine the relationship between changes in cortisol concentrations in different substrates following activation of the hypothalamic-pituitary-adrenocortical axis. The stress model used was claw trimming in a walk in crush.

Materials and methods: The present study was conducted between April 2013 and March 2014. Forty three pluripar German Holstein cows were used (age $4,9 \pm 1,3$ years, milk yield $34,4 \pm 7,2$ kg/day, body weight 626 ± 70 kg).

The concentration of cortisol was measured over a time of ten days, where blood cortisol is seen as gold standard. For repeated blood sampling an indwelling venous catheter was introduced into the left jugular vein. Samples of blood, saliva, lacrima and faeces were taken daily between 08 am – 10 am, excluding day four, at this day the samples were collected between 05 am – 06.30 am. During the daily milking between 1 pm – 2 pm the milk samples were taken.

During claw trimming on day four blood and lacrima samples were taken (minute 0, 15, 25, 30, 40, 60, 80). Faecal samples were measured at minute 480, 540, 600 and 660, respectively. Each time, blood was drawn first from the indwelling catheter and afterwards lacrima samples were taken.

Results: Measuring cortisol in saliva, lacrima and milk and the metabolites in faeces should be a non-invasive method for the widely used blood sampling. Milk sampling is only possible at the daily milking. Normally it is no problem taking faeces without handling the animals, in a dairy farm with about 70 animals per group the unambiguous assignment may be a problem. Without fixation of the animal it is not possible to collect saliva and lacrima.

Cortisol levels measured in the current study are similar to those in the literature. The blood cortisol shows a decrease from day one to day two. This decrease is also shown in lacrima, saliva and faeces. Blood cortisol concentration shows an increase on day five, which is also shown in saliva and faeces. This increase is under the blood cortisol level from

day one. The concentrations from day ten in blood, lacrima, saliva and faeces are under their levels from day one. Milk concentration shows ups and downs, with spikes at day two, four, seven and ten which are always higher than the concentration on day one.

During claw trimming on day four there is an increase in blood cortisol concentration which is also shown in lacrima cortisol.

Conclusions: In this study lacrima, saliva and faeces show the blood cortisol trend on some days. Only milk collection during milking is possible without handling the animals more as you need for a blood sampling. In conclusion, none of the tested body fluids caused less stress than drawing blood from an indwelling jugular catheter.

National Animal Health Programmes

P04-004-049

Suitability of BVD antibody ‘spot testing’ in Irish beef herds as a means of annual surveillance.

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Objectives: Virus testing of a herd, tissue-tag testing of calves, bulk milk antibody testing, and ‘spot-testing’ of youngstock can be used to determine the bovine viral diarrhoea (BVD) status of a herd. ‘Spot-testing’ involves serological testing of 5-10 homeborn, unvaccinated juveniles over 9 months of age. Positive antibody readings in this herd sub-sample can be indicative of BVD persistently infected animals (a PI herd). The objective of the current study was to investigate the usefulness of ‘spot-testing’ as a means of BVD surveillance in beef herds, following completion of the tag-testing phase of the Irish BVD eradication scheme.

Materials and Methods: Blood samples were collected from BVD unvaccinated youngstock in 146 beef farms nationally between November 2014 and February 2015. It was planned to collect a minimum of five samples from weanlings between 9 and 18 months of age, although this was not achievable in all cases. Serum samples were subsequently tested using a BVD virus total antibody ELISA (IDEXX, USA). Samples with an S/P value greater than or equal to 0.3 were classified as positive. Inconclusive results were classified as negative (n=18). The date of birth of each animal sampled and the number (if any) of PI calves born in a herd in 2014, was downloaded from the Irish Cattle Breeding Federation (ICBF) database.

Initially, a herd recording at least one seropositive animal was deemed ‘spot-test’ positive. Data were then reviewed to remove any ‘spot-test’ positive herds that did not meet sampling criteria (i.e. not all youngstock were homeborn or were under or overage) and the dataset reanalysed (‘edt-spot-test’). Finally, a re-classification (‘reclass-spot-test’) was also applied to the edited dataset which categorised herds as ‘spot-test’ positive only if more than one seropositive individual was recorded. Receiver operating characteristics (ROC) analysis was completed using ‘spot-test’, ‘edt-spot-test’, and ‘reclass-spot-test’ datasets in Stata (Version 12, USA). This enabled the usefulness of ‘spot-testing’ in late 2014/early 2015 to identify a PI herd in 2014. For this analysis, birth of at least one PI was the reference variable and ‘spot-testing’ the classification variable.

Results: Of the 146 herds sampled, 94 were ‘spot-test’ negative and did not record a PI birth in 2014. A total of 37 herds were ‘spot-test’ positive and did not record a PI birth, and two ‘spot-test’ negative herds recorded a PI birth. Thirteen herds were ‘spot-test’ positive and generated a PI in 2014. Following dataset edits, 26 herds were excluded (‘edt-spot-test’). Finally, eight herds containing only a single seropositive in ‘edt-spot-test’ were reclassified as negative herds. ROC results for each dataset are included in Table 1.

Table 1. ROC analysis including measures of sensitivity and specificity of ‘spot-testing’ in identifying BVD PI herds.

Category	AUC (95% CI)	Sensitivity	Specificity
‘Spot-test’	0.7921(0.6950, 0.8892)	86.7%	71.8%
‘Edt-spot-test’	0.8565 (0.7421, 0.9708)	83.3%	87.9%

‘Reclass-spot-test’	0.8919 (0.7798, 1.0000)	83.3%	95.1%
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AUC = Area under the curve.

95% CI = 95% confidence interval

Conclusions: If sampling criteria are strictly adhered to, ‘spot-testing’ provides a useful means of identifying beef herds in which a BVD PI has been born. If introduced as a surveillance method, therefore, it is essential to inform sampling personnel of the importance of sampling individuals of the correct age. Specificity of this surveillance method can be improved, without impacting sensitivity, by classifying herds as ‘spot-test’ positive only if two or more seropositives are detected.

National Animal Health Programmes

P04-004-050

Development of Beef HealthCheck – a system for capturing abattoir data on liver and lung lesions in Ireland

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Objectives: Animal Health Ireland (AHI) is a not for profit organisation tasked with pursuing control strategies for economically important diseases of cattle which are not subject to international regulation. In 2015, AHI launched the Beef HealthCheck programme with the aims of developing tools to assist farmers and their veterinary practitioners to control losses due to liver fluke and pneumonia through capture, analysis and reporting of abattoir data and the development by the Irish Cattle Breeding Federation (ICBF) of economic breeding indices that incorporate health and disease data.

Materials and Methods: Beef HealthCheck is an AHI-led programme that has been developed in collaboration with Meat Industry Ireland (MII), ICBF, Veterinary Ireland and the Department of Agriculture, Food and the Marine (DAFM). A standardised Beef HealthCheck scoring system for liver and lung lesions was developed following consultation with AHI’s Technical Working Group on Parasite Control, the DAFM and a review of the literature. AHI has developed material for Temporary Veterinary Inspectors (TVIs), outlining the Beef HealthCheck programme and giving a detailed description of the lesion categories. This material is used as the basis for information meetings for TVIs in each meat factory prior to the roll out of the project. Beef HealthCheck uses touchscreen technology to allow TVIs, who carry out post mortem inspections in Irish meat factories, to record their findings on liver, lungs and where factory layout permits, pregnancies. The data is then transmitted from the meat factory to the ICBF database. Standardised reports for farmers on each batch of animals presented to a factory were developed and are issued directly from meat factories. Over time, farm level reports, reports for veterinary practitioners, and regional reports will also be developed and made available through the ICBF website.

Results: The Beef HealthCheck programme has been rolled out in meat plants across Ireland. Seven large meat processors are involved in the programme and when complete the Beef HealthCheck programme will be receiving information on approximately 80% of the cattle slaughtered in Ireland. Farmers are receiving Beef HealthCheck standardised batch

level reports on the liver and lung lesions found in their cattle at slaughter. Data is accumulating in the ICBF database where it will be used to provide further reports to farmers and their veterinary practitioners and in the further development, by ICBF, of economic breeding indices. The information captured will also allow the effects of liver fluke and pneumonia on a range of production parameters to be measured and economic impact determined.

Conclusions: Liver and lung lesions can negatively influence the carcass weight of cattle at slaughter, the time it takes cattle to reach slaughter weights, milk production and fertility. Beef HealthCheck represents a functional system for capture of information from abattoirs in a standardized way and its presentation to farmers and their veterinary practitioners. This allows them to monitor the health of cattle on farms and to implement control strategies to manage liver fluke, liver abscesses and pneumonia. In addition, it provides the basis for developing herd-level reports and monitoring and forecasting of regional and national trends.

National Animal Health Programmes

P04-004-051

Systematic framework for control and eradication of endemic infectious diseases in cattle

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Objectives: Successful control and eradication of multiple endemic cattle diseases have been achieved in some countries, whereas elsewhere programmes have not been established, despite the required biological knowledge being in place. The objectives are: 1) to describe the individual elements of a systematic control and eradication framework, and 2) to discuss how the individual elements can be compiled for deciding, planning and carrying out effective control or eradication of endemic diseases in cattle, and 3) to illustrate the effect on three infectious diseases in the Danish cattle population.

Materials and Methods: Overall the evidence was established by integration of scientific results with the practical experiences obtained for three diseases over a two-decade period. Thus, published and unpublished data and methods used in the Danish control and eradication programmes for the diseases caused by bovine virus diarrhoea virus (BVDV), Salmonella Dublin (S. Dublin) and Mycobacterium avium subsp. paratuberculosis (MAP) were compiled and grouped into elements evaluated to be needed in an effective control or eradication programme, and the consequential change in prevalence over time was illustrated.

We address the questions: Why is it important or relevant to control or eradicate a specific infection? What should be done to control and eradicate it? Which knowledge gaps exist? Who should be involved and informed, and how should the programme be organised? Where should the programme be carried out in different phases of the programme? Which measures should be used to monitor progress? And when is control and eradication deemed to be achieved?

The basic idea is to enable "disease profiling", which is partially governed by the infectious agent characteristics and its interaction with the host and environment, partially by the impact on, and interaction with, society. It is acknowledged that the necessary information for a sufficient profile often evolve over many years. The profile makes it transparent on which ground the decision is made and how the disease can be addressed.

Results: The key elements include socioeconomic aspects such as establishment of needs and characterisation of motivation for carrying out the programme, animal and veterinary science including assessment of biosecurity and purpose specific test-strategies, organisational issues such as preliminary assessment of programme elements in pilot projects, considerations on resource allocation, administrative and logistic matters, along with aspects such as training, education and communication in general.

All programmes started out with a voluntary control phase supported by surveillance for herd classification and animal testing for management of the infection within herd. For BVDV and S. Dublin extensive surveillance, multiple legislative orders defining control measures and regionalisation, and several pilot projects and communication campaigns were essential parts of the eradication efforts.

The BVDV-programme was initiated in 1994, where the dairy herd level prevalence was 39%. Eradication was achieved in 2006, and all cattle herds are under continuous surveillance for BVDV. The S. Dublin programme was initiated in 2002 at a dairy herd level prevalence of 26%, which today is reduced to 7% and strict legislation applies to prevent spread between herds. The voluntary paratuberculosis control programme began in 2006 when an estimated 85% dairy herds were infected. The main goal of the programme was to reduce the within-herd prevalence, and hence the consequences of the infection. The median within-herd test prevalence in enrolled herds has decreased from approximately 10 to 3%.

Conclusions: We have compiled critical information of generic relevance for effective control and eradication of loss-provoking endemic infectious diseases in cattle into a framework that allows for a systematic, resource-effective approach to combatting endemic infectious diseases. We anticipate the systematic framework approach to be of great interest to an audience interested in National Animal Health Programmes in the cattle sectors.

National Animal Health Programmes

P04-004-052

Livestock Disease Surveillance in Rural Southwestern US, Ten Years of ALIRT

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Objectives: To develop properly trained and equipped first responders, to provide diagnostic support and educational outreach to livestock owners. To develop a systematic approach for sample collection in livestock disease outbreaks and to fully utilize all biological disciplines within a land grant university.

Materials and Methods: The underlying events and reasons for the development of the ALIRT program will be presented. Materials used in training and equipping ALIRT First Responders, ALIRT response protocols and training materials for livestock owners will be presented. A typical ALIRT response will be presented. The ALIRT program has sponsored three national and international meetings regarding livestock disease response and investigation, highlights of these meetings will be presented as well as future plans involving veterinary medical education

Results: The ALIRT program has a ten year history of innovative development of training and response in dealing with livestock losses in the southwestern United States. The ALIRT program can provide a

template for training veterinary students, extension agents, and livestock owners on how to develop a response plan for investigation, diagnosis and response to livestock losses. The ALIRT field investigation protocol provides a template for field veterinarians and extension agents in dealing with livestock losses. The ALIRT field protocol can be used to veterinary students in livestock disease outbreak investigation

Conclusions: The ALIRT program can provide a template for training veterinary students, extension agents, and livestock owners on how to develop a response plan for investigation, diagnosis and response to livestock losses.

National Animal Health Programmes

P04-004-054

Effects of lactobacillus inoculants on fermentation characteristics of wilted Alfalfa with orange pulp silage

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Objectives: This study was conducted to document the effects of Lactobacillus buchneri 40788 inoculants (LAB, LALSIL[®]) on the dry matter content (DM), pH, aerobic stability and microbial population in wilted and chopped (2cm) alfalfa (35% DM) with orange pulp (25% DM) silage.

Materials and Methods: Experimental treatments were wilted alfalfa with no additive (control), wilted Alfalfa and orange pulp (1750 g wilted Alfalfa mixed with 750 g fresh orange pulp) treated with LAB for final application rates of 0, 2.5, 5 and 7.5 g LAB inoculant/ton of wilted alfalfa and orange pulp (LAB0, LAB1, LAB2, LAB3 respectively). Experimental treatments were ensiled in triplicate laboratory mini silos for 90d at room temperature. For isolation and enumeration of microorganisms, 11 g of fresh forage or silage was added to 99 ml of sterile 70mM potassium phosphate buffer (pH 7) and agitated for 60 s on the highest setting in a laboratory blender. Three consecutive dilutions were plated in triplicate onto a semi-selective lactobacilli Medium (MRS) for the isolation of LAB, onto nutrient agar (NA) for the isolation of total bacteria, and onto Sabouraud's dextrose agar (SDA) for the isolation of yeasts and molds.

Results: DM was not affected by treatments at the end of the experimental period. The pH of all silages was less than 4.55, but pH was significantly higher for control compared with silages containing orange pulp (p<0.05). Yeasts and molds count on sabouraud dextrose agar for LAB2, LAB3 treatments were lower than other treatments (p<0.05). Inoculation of LAB caused a significant increase in lactobacillus population on MRS agar for LAB1, LAB2, LAB3 treatments (6.0×10⁷, 7.5×10⁷ and 1.0×10⁸ cfu/gr respectively). Total culturable bacteria in nutrient agar showed significant increase in treatments treated with LAB compare to control (p<0.05). Treating alfalfa silage with orange pulp and LAB improved aerobic stabilities of experimental treatments in comparison to LAB0, significantly (144, 192, 200 vs. 56 and 72 h in, LAB1, LAB2, LAB3 compare to LAB0 and control, respectively).

Conclusions: These findings show that LAB can improve the aerobic stability and quality of silage in laboratory silos and that might orange pulp and LAB improve silage quality and cause better management of silage in the farm.

National Animal Health Programmes

P04-004-055

SURVEILLANCE OF BOVINE ENCEPHALITIS: PAPILOMAVIRUS AS CASUAL AGENT (PRELIMINARY RESULTS)

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Objectives: Bovine encephalitis are generally fatal diseases, with impact in food safety. The Biological Institute of São Paulo State collaborates with the Brazilian national program for surveillance of bovine neurological syndrome, performing the differential diagnosis. The Bovine Papillomavirus (BPV) causes papilloma in skin, and tumors in the digestive and genital systems, but experimental inoculation of BPV into calves' meninges caused papillomatosis in brain. This research aimed to study the frequency of cattle with neurological syndrome, who presented histopathological lesions and BPV DNA in central nervous system (CNS).

Materials and Methods: This research was approved by the Bioethics Committee in Animal Research of the Biological Institute (CETEA-IB), registry number 134/14. A total of 300 CNS samples from several Brazilian regions, in the period from January 2012 to September 2015, were submitted to differential diagnosis of neurological syndrome. There were selected negative samples to rabies (direct immunofluorescence), Neospora caninum, BVDV, BoHV-5, and catarrhal malignant fever (PCR). CNS tissues were fixed in buffered formalin 10%, and submitted to histological analysis using hematoxylin-eosin staining in paraffin embedded tissue. For BPV analysis, DNA was extracted from refrigerated samples by Guanidinium thiocyanate-phenol-chloroform method. The amplification of the DNA fragment was performed by generic primers, with nucleotide sequence of the gene that codifies the L1 protein of the BPV capsid: FAP59 (5'-TAA CWG TIG GIC AYC CWT ATT-3'), and FAP64 (5'-CCW ATA TCW VHC ATI TCI CCA TC-3'). The negative BPV PCR control was ultra-pure water, and the positive BPV PCR control was a BPV-6 DNA from a skin papilloma diluted 1:10). The mix used in the PCR was: 12.5 µL Master Mix Promega®, 3.75 µL Nuclease Free Water, 1.875 µL each primer diluted 10 pmol, and 5 µL of DNA sample. The program of amplification consisted in initial denaturation 94°C/5 minutes, 45 cycles of amplification (94°C/1 minute; 49.3°C/1 minute; 72°C/1 minute) and final extension 72°C for 5 minutes. The samples were considered positive when they presented a band corresponding 478 bp with equal or stronger similarity to the positive control.

Results: It was found 4.3% (13/300) CNS samples positive to BPV PCR. In 11 of these 13 positive samples (84.6%), there was no histopathological alterations in CNS, and 2 (15.4%) had typical lesions of non-purulent meningoencephalitis. Considering the gender, 69.2% of the positive samples were female, and 30.8% male. The animals were aged from 5 to 168 months old, with a higher frequency of positivity from 24 to 36 (30.8%). In the positive cases, 38.6% were dairy cattle, 30.7% crossbreed, and 23% beef cattle. The low number of positive BPV samples didn't allowed the statistical analysis among PCR positivity, histopathological lesions, gender, and breed. The positive BPV DNA samples had no papilloma, but presented unspecific inflammatory non purulent lesions.

Conclusions: These preliminary results suggest low occurrence of BPV in natural infection in CNS of cattle with neurological syndrome. The next step of this study will be the genome sequencing of the positive samples, in order to conclude the frequency of BPV, and to know what genotype of BPV is being found in the CNS. The viral phylogenetic tree will be

constructed for understanding the molecular epidemiology of BPV in the Brazilian bovine population.

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National Animal Health Programmes

P04-004-056

Animal Health Ireland Parasite Control Technical Working Group leaflets on Coccidiosis and Cryptosporidiosis as a method of knowledge transfer.

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Objectives: Animal Health Ireland (AHI) is a not for profit organisation tasked with pursuing control strategies for economically important diseases of cattle which are not subject to international regulation. AHI's Parasite Control Technical Working Group (TWG) aims to produce objective and practical guidelines for farmers, on the control of parasites affecting cattle on Irish farms.

Materials and Methods: The parasite control TWG has produced two new information leaflets: "Bovine coccidiosis – the facts" and "Cryptosporidiosis in neonatal calves", aimed at farmers and their veterinary practitioners. The leaflets have been produced following a thorough review of the literature and discussion among the Parasite Control TWG experts in the fields of parasitology and veterinary medicine. The advice is aimed specifically at Irish farmers and acknowledges the practices and conditions on Irish beef and dairy farms. The leaflets are complementary to other material produced by AHI's Calcare and Biosecurity TWGs. The leaflets contain information on the lifecycles, transmission, clinical signs, diagnosis, treatment and control of these parasites. The leaflets are available through the Animal Health Ireland website.

Results: The leaflets are a vehicle for the transfer of objective, science based knowledge to farmers. Farmers can access the information through the AHI website and the material from the leaflets is also used as the basis for articles that appear in AHI newsletters and the farming press. The leaflets are regularly referenced in material produced by AHI stakeholder organisations and in academic and media articles on parasites. Annually, AHI undertakes a series of information events on Calf health and in the spring of 2016, a section of these events focused on coccidiosis and cryptosporidiosis.

Conclusions: AHI is a trusted and reputable source of factual, up to date and independent information for the agricultural community in Ireland. The leaflets build awareness of coccidiosis and cryptosporidiosis on Irish farms and methods to control and prevent the diseases caused by these parasites.

National Animal Health Programmes

P04-004-057

Evaluation of a young stock check test (YSCT) to detect the presence of BVD persistently infected animals in Irish dairy herds

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Objectives: Several diagnostic strategies may be used to establish the BVD status of a herd including virus testing of all animals, bulk milk antibody testing and young-stock check tests (YSCT). YSCT involves serological testing of a limited number of home-born, unvaccinated juveniles, greater than 9 months of age. Detection of BVD virus antibodies in young-stock is likely due to exposure to one or more persistently infected (PI) calves. Our objective was to evaluate the performance of a YSCT in dairy herds during the current phase of the national BVD eradication programme in Ireland.

Materials and Methods: Samples were collected in 137 dairy herds in the south west of Ireland from September to December 2014 as part of a targeted surveillance programme. Samples from qualifying juveniles stock were tested using a BVD virus total antibody ELISA (IDEXX, USA) having a positive cut-off of ≥ 0.3 S/P (99.5% specificity (Sp) and 96.3% sensitivity (Se)). The Irish Cattle Breeding Federation provided relevant details of all PIs born in 2014 and 2015 (data available from the Irish national BVD eradication programme). The programme records a calf as PI if it has at least one BVD virus positive test without a negative confirmatory test.

Descriptive data were coded and analysed in Excel (MS Office 2010). Statistical analyses were completed using Stata version 12 (StataCorp, US). Non-parametric receiver operating characteristics (ROC) analysis without covariates was applied to serological YSCT (classification variable) and BVD PI data (reference variable) to examine the usefulness of a 2014 YSCT in identifying herds in which a PI calf was born in either 2014 and/or 2015. Two datasets were constructed; herds recording at least one seropositive juvenile were deemed YSCT positive (YSCT1), and herds recording at least two seropositive juveniles deemed YSCT positive (YSCT2).

Results:

Dataset 1

Of the 27 herds in which at least one PI was born in 2014, 20 herds recorded a positive YSCT. Of herds with a PI born in 2015 (n=12), seven were YSCT positive. ROC area under the curve (AUC) values for 2014 and 2015 PI births were 0.78 and 0.66, respectively. Classifying herds on the basis of at least one serologically positive juvenile (YSCT1) yielded a Se and Sp of 74.1% and 80.9% respectively in relation to herds with a PI birth in 2014. In terms of using YSCT to identify herds which may produce a PI in the following calving season, Se and Sp were 58.3% and 72.8% respectively.

Dataset 2

If herds were classified as young-stock positive if two or more juveniles were positive (YSCT2), ROC AUC values dropped to 0.71 for 2014 born PIs and 0.56 for 2015 born PIs. Use of this YSCT2 herd classification, although improving Sp (93.6% for 2014, 86.4% for 2015) over dataset 1, caused a considerable drop in Se (48.2% for 2014, 25% for 2015).

Conclusions: Even though positive herd YSCT Se was low, YSCT1 was still found to be superior to YSCT2 in terms of Se and AUC. The Se of both YSCT1 and 2 in predicting the birth of PIs in 2015 was low. Currently, use of YSCT (particularly YSCT1) has a low Sp, resulting in “false positive” results in 2014 for 24% of herds in which no PIs were born. Future Sp of YSCT is expected to improve as national herd BVD immunity (and therefore the presence of maternally derived antibodies) reduces. Se is also expected to improve, in future, in herds that are no longer routinely tissue tag testing calves at birth.

National Animal Health Programmes

P04-004-058

Correlation between BVD blood and milk serology in first lactation dairy cows.

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Objectives: Serological investigation of individual cows on dairy farms can be used as a surveillance tool to identify circulation of BVD virus. Blood or milk can be used for the purposes of serological testing. Milk may be a preferred sampling option as it is non-invasive and less expensive. The objective of this study was to investigate the correlation between blood and milk samples in first lactation dairy cows to determine if milk samples provide a viable alternative to BVD blood serological testing for herd surveillance.

Materials and Methods: Blood and milk samples were collected between September and December 2014 from five first lactation cows in 102 dairy herds in the south western region of Ireland. Both blood and milk samples were tested using the BVD virus total antibody ELISA (IDEXX, USA). A positive cut-off of ≥ 0.3 S/P was applied and samples yielding S/P ratios of ≥ 0.2 and < 0.3 were classified as inconclusive as per kit manufacturer’s instructions. Results were analysed as both continuous (ELISA readings) and categorical (positive, inconclusive, negative) data. Two herd-level categorical datasets were also constructed. Herds were categorised as positive on the basis of having at least one (herd dataset 1), or at least two (herd dataset 2) positive individuals. Negative status was assigned if either no individuals recorded positive results (herd dataset 1) or only recorded a single positive (herd dataset 2). For statistical analysis, data were sorted and coded in Excel (MS Office 2010) and imported to Stata Version 12 (StataCorp, USA). Paired t-test and Pearson (continuous data) and chi-squared and Spearman (categorical) correlation analyses were completed.

Results: Of the 102 herds studied, approximately 80% were spring-calving. A total of 47 herds vaccinated for BVD. The average lactating herd size was 69 cows (range 20-220) which is reflective of the national average. Blood and milk results were available for a total of 510 cows. Spearman and Pearson correlation analyses of individual cows yielded rho values of 0.74 and 0.87 indicating good agreement between both sample matrices. The mean S/P ratios were 0.32 and 0.52 for milk and blood respectively and significantly different ($P < 0.001$). Pearson chi-squared also highlighted a significant difference ($P < 0.001$) between

milk and blood categorical data. A total of 39 blood seropositives were classified as negative using milk samples, and 16 milk seropositives were classified as negative on blood. At a herd level, Spearman correlation rho values for herd datasets 1 and 2 were 0.71 and 0.78, respectively. Herd dataset 1 yielded seven herds that were milk positive but blood negative, and six herds that were blood positive and milk negative. Using herd dataset 2, 10 herds were blood positive and milk negative with two herds milk positive and blood negative. Chi-squared analysis of milk versus blood herd categorisation yielded P values of < 0.001 .

Conclusions: Although a strong correlation was identified between milk and blood samples analysed as both continuous and categorical data, the discrepancies between blood and milk categories at both individual animal and herd level may indicate either sensitivity or specificity issues with these sample types. Further investigation is required to elucidate whether blood analysis is less-specific than milk or milk analysis is less sensitive than blood. Data are available on the BVD viral status of each herd and further investigations are underway which should allow determination of the most appropriate surveillance sample on Irish dairy farms

Neosporosis

P04-004-167

The Importance of Fetal Pathology for Neosporosis Diagnosis and Control

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Objectives: *Neospora caninum* has been recognized as a significant cause of bovine abortions worldwide, although a definitive diagnosis of *N. caninum* is a major challenge. Furthermore, epidemiologic findings are non-specific, as abortions can occur anytime in gestation and can be observed in an epidemic or endemic pattern. Therefore, the objectives of this study were to determine causative agents of bovine abortions and describe the prevalence and risk factors associated with *N. caninum* abortions in BC Animal Health Center (AHC) submissions retrospectively. Additionally we identify risk factors associated with fetal abortions.

Materials and Methods: Between January 2007 and July 2013, there were 182 bovine fetal submissions and from July 2013 to May 2014 active case recruitment was initiated from producers through the local veterinary clinic, resulting in another 54 abortion submissions.

Fetuses presented for necropsy underwent a standardized post mortem examination and cases were confirmed by histopathology after initial positive test results in either immunohistochemistry, serology (cELISA, Pullman, WA), or polymerase chain reaction for Apicomplexa (Np21/Np6 and ApilTS1 primers). We set a case definition for *Neospora* and performed a logistic regression finding associations between fetal Neosporosis and risk factors like breed, age, parity, season, gestation length and location.

Results: A diagnosis could be made in 64.8% of all submissions, with a confirmed etiology identified in 41.1% of submissions; 15.3% bacterial, 15.3% protozoal, 4.2% viral, 1.7% developmental, 0.8% fungal, and 3.8% mixed infections. Neosporosis was confirmed in 19.1% of all submitted fetuses from 2007-2014 and myocarditis, myositis and encephalitis were the most common microscopic findings. Between January 2007 and July 2013, 14.4% of the abortions were diagnosed as neosporosis. In contrast, with active case recruitment of fetal abortions between July 2013 and May 2014, the incidence of neosporosis increased to 40.9% of the submissions. Neosporosis was most commonly identified in fetuses between 3 to 6 months gestation and there was no significant association with dam parity. Some differences in diagnoses may be attributed to test sensitivity and specificity. Active surveillance established that immediate submission of bovine abortion samples dramatically improved diagnosis and detection of *Neospora caninum* as a significant causal agent of bovine abortion and production losses in the upper Fraser Valley(BC), a region where neosporosis is highly prevalent.

Conclusions: *N. caninum* is the most commonly diagnosed infectious etiology associated with bovine abortions. While definitive diagnoses of *N. caninum* remains challenging, use of multiple diagnostic tools including fetal pathologic examination, pathogen identification by PCR, IHC and serology, combined with epidemiological data, can provide valuable information for pathologists, veterinarians and producers. Active surveillance could also increase understanding of *N. caninum* epidemiology. With known causes of bovine fetal losses and abortions, better focused strategies to mitigate the economic impact of fetal losses can be developed.

Neosporosis

P04-004-168

Seroprevalence of *Neospora caninum* infection in dairy cattle in central and northeastern Poland

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Objectives: A cross-sectional study was carried out to characterize the seroprevalence of *Neospora caninum* infection in cattle in central and northeastern Poland and investigate the relationship between grazing policy and *N. caninum* infection.

Materials and Methods: Ninety seven dairy cattle herds from 2 provinces of Poland (Podlaskie and Łódzkie) were randomly enrolled in the study. Sample size from each herd ranged from 3 to 16 dairy cows with median of 8 (interquartile range from 6 to 9) and a total number of 734 cows were enrolled. Moreover, 1 to 5 calves (<18 month-old) were randomly selected="selected" in 61 of 97 herds (175 calves in all). The animals were screened with a commercial competitive ELISA (Bio-X Diagnostics, Belgium). Then, all 909 tested animals were classified into 5 age categories: <18 months (n=175), 18-36 (n=214), 37-60 (n=285), 61-84 (n=156), >84 (n=79) and true individual-level seroprevalence was calculated for each of them. To calculate true-herd level seroprevalence the test sensitivity and specificity were adjusted from an individual- to a herd-level using FreeCalc method.

Results: The true herd-level seroprevalence of *N. caninum* infection was 56.7% (95% CI: 46.8%, 66.1%). One hundred forty three of 734 cows (19.5%) were seropositive which gave the true overall individual-level seroprevalence of 20.1% (95% CI: 17.4%, 23.2%). Percentage of seropositive cows in each herd varied from 6% to 80%. No difference between seropositive and seronegative herds was found with respect to the median herd size (21.5 vs. 21.0 dairy cows; p=0.645), grazing policy (full-day: 42.9% vs. 45.0%, half-day: 8.9% vs. 12.5%, no grazing: 48.2% vs. 42.5%, respectively; p=0.690) nor grazing season length (median of 5.0 months in both herd groups; p=0.445). Individual-level seroprevalence proved to increase along with animal age (p=0.018).

Conclusions: *Neospora caninum* is a protozoan of two-host life cycle with a domestic dog or another representative of Canidae family as a definitive host. To date, wide range of animal species susceptible to *N. caninum* has been identified, with cattle of the highest economic importance. The infection and *Neospora*-associated bovine abortion have already been reported from virtually all European countries. Herd-level seroprevalences varied from below 1% (Norway) to almost 90% (Spain). As seen in this study, *N. caninum* infection is widespread in Polish dairy cattle population and thus has to be considered as a potential cause of spontaneous abortions.

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Neosporosis

P04-004-169

Horizontally or Vertically Infected Dairy Cattle with *Neospora caninum*: rate of transmission to offspring and serological characterizationRaphaël Guatteo¹ Emma Dupuis¹ Justine Boyer¹ Alain Joly² Christophe Chartier¹ Caroline Lantuejoul³ Grégoire Kuntz⁴¹UMR 1300 Oniris & INRA BioEpAR, Nantes, ²GDS Bretagne, Vannes, ³FRGDS Pays de la Loire, Nantes, ⁴GDS Bretagne, Ploufragan, France

Objectives: The principal route for *Neospora caninum* infection is supposed to be transplacental (vertical), the congenitally infected cow passing the infection to multiple offspring. In case of postnatal (horizontal) infection, the dam would pass the infection to the immediate offspring (or abort) and would pass to a lower rate the infection to further offspring. But these reports are based on a small number of experimental studies. The objective was to describe the rate of transmission to the offspring depending on the supposed route of infection and to investigate the value of ELISA test (single or repeated) to discriminate the route of infection.

Materials and Methods: The determination of the rate of transmission to their offspring was calculated based on large dataset (251 548 blood ELISA tests among which 19 547 cattle with at least 2 serological results) coming from the control plans implemented in the western part of France for the last 15 years in dairy herds facing repeated abortions. A cow was supposed to be infected by horizontal route when a seroconversion occurred during the control plan. A cow was supposed to be infected by vertical route if its own dam was already seropositive before its birth and if this cow was seropositive from its first testing (occurring after 6 months of age). Then, the number of offspring detected seropositive or seronegative (after 6 months of age) was compared between two groups using chi-square test. In a second step, a longitudinal follow-up was conducted in 325 dairy cows from 20 dairy herds with repeated abortions and a within herd seroprevalence > 20% suggesting the co-existence of both transmissions routes. Kinetics of serological results over time was compared between supposed infection routes. In a last step, the putative informative value of the optical density of the first positive ELISA tests was investigated using receiver operating characteristic (ROC) method.

Results: After the selection process, only 69 and 60 cows could be considered as infected through vertical and horizontal routes respectively. This small number was related (i) to the quite systematic culling of seropositive animals due to control plans and thus did not make the follow-up of seropositive animals and their offspring easier, (ii) the fact that male calves were not tested. The rate of transmission to the offspring was found significantly different ($p=0,03$) between supposed congenitally infected dam (65%) and supposed postnatal infected ones (47%) confirming a lower transmission rate for horizontal route but to a lower extent that previously described although horizontal contamination of naïve calves after birth could not be fully excluded. Over a 12 months period, less than 8% of supposed vertically infected cows became seronegative while 35% and 44% of supposed horizontally infected ones were seronegative 6 months and 9 months respectively after the first positive result. Lastly with the serological tests used in the follow-up, the mean of S/P ratio were significantly higher for supposed vertically infected dams. The ROC curve indicated a cut-off allowing reaching specificity of 70% and sensitivity of 50% to distinguish supposed horizontally infected dams.

Conclusions: These results, based on a large dataset under field conditions, confirm difference in transmission rate to the offspring depending on the route of infection for the cow but to a lower extent of that was already reported. ELISA quantitative results or serological follow-up could be used, with prevalence and filiations data, to distinguish infection route and therefore prioritize culling.

Nutrition & Metabolic Diseases

P01-001-023

Reduction of detrimental effect of Aflatoxin B1 on In Vitro rumen fermentation by addition of MycosorbZabihollah Nemati¹, Maghsoud Besharati¹, Mojtaba Karimi¹¹Animal Science, university of Tabriz, Tabriz, Iran, Islamic Republic Of

Objectives: The objective of the present study was to investigate the effect of different doses of Mycosorb on in vitro gas production of alfalfa hay based diet and final PH in the cultures after 120-h incubation in the present of 1µg/ml aflatoxin B1.

Materials and Methods: Ruminant liquor was collected after morning feeding from 2 cannulated sheep. Ruminant liquor was immediately filtered through four layers of cheesecloth and filtered rumen liquor was used as a source of inoculum. The resulting ruminal liquor was purged with deoxygenated CO₂ before use as the inoculum. Briefly, 300 mg of diet were weighed in to 50-ml bottles and incubated in buffered rumen fluid for 120 h and the gas production recorded at 4, 6, 8, 12, 24, 48, 72, 96 and 120 h. Experimental treatments were four dose levels of glucomannan (Mycosorb™) at 0, 0.5, 5 and 10 gr/L in the present of 1µg/ml aflatoxin B1. After 120 h incubation, bottles were opened and final pH of culture fluids was measured.

Results: The results showed that cumulative gas production at 24, 48, 72, 96 and 120h after incubation differed significantly. The lower volume of gas production was observed at the zero dose of Mycosorb in the present of aflatoxin B1. Mycosorb addition affected the rate and cumulative gas production so by increasing the level of Mycosorb from 0 to 10 gr/L, the cumulative gas production increased from 389 to 692 ml/g dry matter, respectively ($p < 0.05$). Final culture pH was significantly affected by aflatoxin B1 binder. The pH was decreased significantly due to addition of Mycosorb at the concentration of 5 or 10 gr/L ($p < 0.05$).

Conclusions: It was concluded from the present study that addition of Mycosorb improves rumen metabolism and has potential to reduce detrimental effect of Aflatoxin B1 on rumen fermentation of diet. This product can be incorporated in to animal feed to prevent disease associated to aflatoxicosis.

Nutrition & Metabolic Diseases

P01-001-024

Impact of Virginiamycin used as Growth Promoter on the Ruminant Microbiota of Feedlot CattleBruna P. Santos¹, José A. Bessegatto¹, Lais R. Paulino¹, Julio A. Lisboa¹, Amauri A. Alfieri¹, Carlos H. Montemor¹, Scott Weese², Marcio Costa^{1,2}¹Universidade Estadual de Londrina, Brazil, Londrina, Brazil,²University of Guelph, Guelph, Canada

Objectives: The ruminal microbiota promotes digestion and production of volatile fatty acids, the main energy source for ruminants. The composition of ruminal microbiota may be related with fermentation efficiency, and can be modified by various factors. The mechanisms by which antibiotic growth promoters (AGPs) modify the ruminal microbiota to increase feed conversion rates have not been fully elucidated. The aim of this study was to compare the microbiota in the rumen of beef cattle receiving virginiamycin with a control group at the exit from a feedlot operation.

Materials and Methods: Fifty crossbred beef cattle (26 females and 24 males) housed in a feedlot operation were enrolled. Animals were divided in two groups: ATB (n=25), in which animals received virginiamycin (340mg/360Kg body weight) and CON (n=25), in which animals received the same diet, but without any AGP. Slaughter was carried with 66, 88 or 116 days of confinement, depending on their body score condition. Ruminant fluid samples were taken during slaughter directly from the medial portion of the rumen, and immediately refrigerated until frozen at -80°C. DNA extraction was performed using a commercial kit and the V4 region of the 16S rRNA gene amplified by PCR and sequenced with an Illumina MiSeq platform. The different species composing the microbiota (community membership) and the bacterial composition taking into account the evenness of each species (community structure) were addressed respectively by the Classical Jaccard and the Yue and Clayton indices. A P-test was used to compare membership and structure and a t test to compare relative abundances found within each group.

Results: Community membership and structure present in the rumen of animals treated with virginiamycin were statistically different from ones found in the control group ($P=0,001$ and $P=0,005$, respectively). However, no significant differences were observed in the relative abundance of the main phyla and genera (all $P>0.010$), suggesting that changes were likely more evident in less abundant species. More than 50% of bacteria present in the rumen of both groups were classified as Firmicutes. The second most abundant phylum was Bacteroidetes followed by Proteobacteria, Fibrobacteres, Verrucomicrobia and Spirochaetes.

Conclusions: The use of virginiamycin as AGP significantly affected membership and structure of bacterial communities present in the rumen of feedlot cattle at the time of slaughter.

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Nutrition & Metabolic Diseases

P01-001-025

An assessment of the effectiveness of a hypocalcaemia prevention protocol on Irish dairy farms during the spring of 2016Maresa Sheehan¹, Colm Brady¹, John Fagan², Seamus Fagan², Denise Murphy², William Fitzgerald¹, Joe Patton³¹Department of Agriculture, Food and the Marine, Regional Veterinary Laboratory, Hebron Road, Kilkenny, ²Department of Agriculture, Food and the Marine, Regional Veterinary Laboratory, Coosan, Athlone, Co Westmeath, ³Teagasc, Grange Research Centre, Dunsany, Co Meath, Ireland

Objectives: To advise and help to implement a protocol for the prevention and control of clinical and subclinical hypocalcaemia in twelve selected dairy herds that have a history of hypocalcaemia related problems, including milk fever, prolonged parturition, slow weak calves, retained foetal membranes and metritis. An assessment of the protocol's effectiveness will be made by measuring the number of hypocalcaemia related events that occur on the study farms and comparing them to farm records for 2015.

Materials and Methods: The study farmers completed a questionnaire detailing the number of hypocalcaemia related events (e.g. milk fever, slow calvings, weak calves, retained foetal membranes, metritis) and hypocalcaemia prophylaxis used on their farms in spring 2015. Details of body condition score (BCS), silage analysis, mineral supplementation

and any additional components of the dry cow diet were collected for each farm in November 2015. Based on this information an individual protocol document was issued to each farm with the aim of achieving the following targets; BCS of 3.25 at calving, 0.40% magnesium, 0.3% phosphorous, and a maximum of 2.0% potassium in the diet DM for late gestation dry cows. Blood samples will be collected from ten cows four weeks prior to start of calving and again when the first six cows have calved to evaluate the success of implemented measures by measuring serum metabolites including calcium and magnesium levels. Private veterinary practitioners (PVPs) have been instructed to take a blood sample from any periparturient cow (within 48 hours) that he/she attends on the study farms during spring 2016. The incidence and treatment of conditions associated with hypocalcaemia and the use of hypocalcaemia prophylactic treatment will be recorded by the farmers. All perinatal mortality on the study farms will be submitted to the local Department of Agriculture and the Marine (DAFM) laboratories for post mortem examination.

Results: The incidence of hypocalcaemia related conditions on these farms, the use of hypocalcaemia prophylactic treatment and the results of blood samples will be collated for each farm. The success of the protocol will be assessed by comparing these results to 2015 farm records. The specific measure that will be used will vary from farm to farm depending on the particular hypocalcaemia related issue the farmer experienced most in 2015. Therefore on some farms the incidence of milk fever will be used while on another the incidence of prolonged parturition will be more appropriate. Based on the study findings and the practical experience of the farmers, the protocol will be refined accordingly to be applicable for dairy farms in Ireland.

Conclusions: We aim to publish the advice as a joint DAFM Laboratories/ Teagasc protocol for use by private veterinary practitioners and Teagasc Advisers to prevent subclinical and clinical hypocalcaemia on farms. It is hoped that the results of this study may generate further areas of research and collaboration between Teagasc, DAFM laboratories and PVPs for the improvement of Irish Agriculture.

Nutrition & Metabolic Diseases

P01-001-028

Effects of chemical treatment of barley with tannic acid or alum on blood metabolites and productive performance of lactating Holstein dairy cow

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Objectives: Barley grain is poorly utilized by ruminants due to its rapid degradation in rumen. Barley processing is imperative to maximize its utilization by feedlot and dairy cattle. The objectives of this study were to evaluate the effects of chemical treatment of barley with tannic acid or alum on blood metabolites, dry matter digestibility and productive performance of lactating Holstein dairy cow.

Materials and Methods: An experiment was conducted to evaluate the effects of chemical treatment of barley grain with tannic acid and alum (KAl(SO₄)₂) on blood metabolites and productive performance of lactating Holstein dairy cow. In this experiment a completely randomized design with 18 cows (average milk production, 34.7 kg/d), three treatments and 6 cows per each treatment were employed. The experimental diets 1 through 3 contained untreated barley, barley treated with tannic acid (5% weight), and Alum (5% weight) respectively. The cows were fed

individually ad libitum three per day. Daily milk yield was recorded and samples of the milk were taken three times per week, also samples of rumen liquors, urea and blood were taken in end of period.

Results: There were significant differences (P<0.05) between treatments in dry matter intake. Diets containing barley treated with tannic acid had the highest dry matter intake and difference were significant (P<0.05) between treatments. In this experiment body weight change and fat corrected milk of cows were not affected by experimental diets. Fat percent of milk were increased by feeding ration containing barley treated with tannic acid (p<0.05). The concentration of lactose, protein and solid non fat in milk were not affected by rations. The pH of cow's rumen liquor and urea were significantly different between rations. Also concentration of glucose, calcium, phosphorus, sodium, cholesterol, triglycerides, magnesium, potassium and total protein in blood plasma of cows received different diets were not different (p>0.05) but blood urea nitrogen and glucose concentrations of cows received diet containing barley treated with tannic acid were higher than other treatments.

Conclusions: On the basis of these data, we can conclude that feeding barley treated by tannic acid or Alum increase dry matter intake and milk yield.

Nutrition & Metabolic Diseases

P01-001-029

Red Blood Cell Profile And Oxidative Stress In Cattle Feedlot Fed With Brachiaria Sp Hay And Supplemented With Antioxidants

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Objectives: Brachiaria sp species contain sporidesmins, which lead to lipid peroxidation and oxidative stress due oxidation. The aim of the present study was to evaluate the effects of different antioxidants in Nelore cattle fed with Brachiaria sp in the prevention of lipid peroxidation in cell membranes.

Materials and Methods: The study was conducted at the Veterinary School's farm named Tomé Pinto of the Federal University of Goiás, in São Francisco de Goiás, GO. A total number of 40 animals were divided into five groups being confined for 105 days. The experimental design was completely randomized with five treatments: Group 1: non supplementation – control; Group 2: supplementation of selenium and vitamin E (10 mg of selenium and 1000 IU of vitamin E / animal / day); Group 3: zinc supplementation (600 mg zinc / animal / day); Group 4: selenium supplementation (10 mg selenium / animal / day) and Group 5: vitamin E supplementation (1000 IU vitamin E / animal / day). Animals were fed twice a day and had access to water ad libitum. The diet followed the proportion of 70:30 for forage and concentrate, respectively. Blood samples were harvested by venous puncture. The samples were stored in heparinized tubes and then analyzed. To evaluate oxidative stress, erythrocyte membrane characterization (red blood cells, hemoglobin and packed cell volume), TBARS, GSH-T, GSH-Px, SOD and CAT were performed.

Results: The parameters analyzed in erythrogram not influenced by the different supplementation regimens adopted throughout the experiment (p>0.05). Among the lipid peroxidation markers, there was statistical difference between the treatments proposed for the measurement

of TBARS ($p < 0.05$) in the animals who received supplementation of selenium and vitamin E showed greater lipid stability. The other oxidative stress markers present numerical variations between the standard treatment, but this variation did not provide biological changes in animals.

Conclusions: No oxidative stress was detected during the experimental period when animals were confined even when using antioxidants. Additionally, selenium and vitamin E combined in same diet seemed to cause less deleterious alterations on the erythrocyte.

Nutrition & Metabolic Diseases

P01-001-031

Can a natural growth promoter improve the health of dairy calves?

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Objectives: An increasing number of antibiotic-resistant pathogens is currently being discovered in conventional dairy production. Practices such as applying antibiotics for prophylactic health benefits allow pathogens to build up resistances (Call et al., 2008). A broad range of alternative substances, such as phytochemicals, organic acids and salts thereof, can, however, also inhibit pathogenic bacteria. The aim of this feeding trial is to test the dosage-dependent effects of a product consisting of salts of organic acids, a phytochemical, and a permeabilising substance (Biotronic® PX Top3) on the health and performance of Holstein calves.

Materials and Methods: All animals were allocated to four groups, one control with standard diet only and three groups treated with the product Biotronic® PX Top3: Standard diet + 3.0 g product per calf per day in milk for ten weeks, standard diet + 3.0 g product per calf per day in milk during weeks one to four and 1.5 g product per calf per day in milk during weeks five to ten, or standard diet + 6.0 g product per calf per day in milk for ten weeks.

The following parameters were assessed weekly: Body weight, faeces consistency; nasal and ocular discharge scores, and coughing scores. Independent samples Kruskal-Wallis-tests were carried out using SPSS 19.

Results: Lower scores, indicating improved health, as well as increased average daily weight gains were recorded in all treated groups: Total average nasal and ocular discharge, coughing, and faeces consistency scores in the control group (nose 0.72, eyes 0.63, coughing 0.60, and faeces 0.90) were significantly higher than those in the treated groups (nose 0.30-0.32, eyes 0.22-0.26, coughing 0.12-0.23, and faeces 0.32-0.40 respectively), with $p < 0.05$.

The average daily weight gain was also significantly ($p < 0.05$) improved by the product: The untreated group showed an average daily weight gain of $701 \text{ g} \cdot \text{d}^{-1}$, whereas the calves treated with 6.0 g of the product per day gained $838 \text{ g} \cdot \text{d}^{-1}$.

Conclusions: The product (Biotronic® PX Top3) shows beneficial effects on the health of calves in all three concentrations: It significantly reduced nasal and ocular discharge as well as coughing ($p < 0.05$). Furthermore, it improved the consistency of faeces significantly ($p < 0.05$) and the average daily weight gain could be increased significantly ($p < 0.05$) in the group treated with 6.0 g of product per day and calf.

Reference: Call, Douglas R.; Davis, Margaret, A., and Ashish A. Sawant. (2008). Antimicrobial resistance in beef and dairy cattle production. *Animal Health Reviews* 9 (2); 159-167.

Nutrition & Metabolic Diseases

P01-001-032

Effects of prolonged consumption of water with elevated nitrate levels on certain biochemical parameters of dairy cattle and use of clinoptilolite

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Objectives: Elevated levels of nitrates in feed and water can pose a significant health risk for dairy cattle. The objective of the present study was to investigate the effect of prolonged consumption of water naturally contaminated with nitrates on certain biochemical parameters in dairy cows. Concurrently, it was further examined whether the in-feed inclusion of clinoptilolite, a natural zeolite with high selectivity for ammonia cations, could ameliorate nitrate consumption consequences.

Materials and Methods: Two experiments were run simultaneously for the purpose of the study. In the first experiment (Exp.1) farms 1 and 2 were assigned into two groups according to the nitrate levels in the borehole water of the previous year. The first group (NG) consisted of the farm 1 (milk yield; MY 35 kg/cow/day) that had nitrate levels > 40 ppm and the second one (CG) of the farm 2 (MY 36 kg/cow/day) that had water nitrate levels < 40 ppm. In the second experiment (Exp.2) farms 3 and 4 were assigned into two groups according to the nitrate levels in the borehole water and the use of clinoptilolite as follows: the first group (NC) consisted of the farm 3 (MY 31 kg/cow/day) that had nitrate levels > 40 ppm and used clinoptilolite as feed additive at the rate of 2.5% of concentrates. The second one (CG) consisted of the farm 4 (MY 29 kg/cow/day) that had water nitrate levels < 40 ppm without adding clinoptilolite in the ration. Both experiments lasted 6 months. Each farm was visited 3 times, at the onset of the experiment and then at 3-month intervals. At each visit, 15 clinically healthy dairy cows, 5 early lactation cows (< 150 days in milk; DIM), 5 late lactation cows (≥ 150 DIM) and 5 dry cows were selected randomly for blood sampling. Blood glucose and beta-hydroxybutyrate (BHBA) levels were determined on farm with a portable device. In serum, the concentrations of BUN, albumins, Ca, P, K, Na, Mg, Fe, Cu and Zn and the activities of AST and γ -GT were determined using an automatic biochemistry analyzer and flame atomic absorption spectrophotometry. The data was analyzed with SPSS® 21.

Results: The average water nitrate levels during the experimental period were 87 ± 12.5 ppm for farm 1, 4 ± 1.5 ppm for farm 2, 13 ± 2.4 ppm for farm 3 and 76 ± 14.2 ppm for farm 4. Blood BHBA concentration was significantly higher in the late lactation cows of the farms with high nitrate levels compared to those with low nitrates in both experiments (Exp.1: 0.78 ± 0.08 and 0.42 ± 0.03 mmol/l for groups NG and CG, respectively, $P < 0.05$; Exp. 2: 0.85 ± 0.06 and 0.65 ± 0.03 mmol/l for groups NC and CG, respectively, $P < 0.05$). Blood glucose concentration was also significantly higher in NG than the controls in the early lactation cows in Exp. 1 (3.13 ± 0.10 and 2.82 ± 0.12 mmol/l for groups NG and CG, $P < 0.05$). Average BUN values in the same experiment were significantly higher in NG compared to CG in all three categories of cows (early lactation: 7.14 ± 0.20 and 5.45 ± 0.41 mmol/l, for groups NG and CG, $P < 0.05$; late lactation: 6.43 ± 0.25 and 5.42 ± 0.26 mmol/l for groups NG and CG,

P<0.05; dry cows: 6.64±0.42 and 3.64±0.16 mmol/l for groups NG and CG, P<0.05). In Exp. 2, BUN concentration was significantly lower in NC group compared to the controls in early (3.45±0.24 and 5.39±0.46 mmol/l for groups NC and CG, P<0.05) and late lactation cows (3.50±0.18 and 4.68±0.38 mmol/l for groups NC and CG, P<0.05) whereas, it was unaffected in dry cows (3.48±0.40 and 3.82±0.39 for groups NC and CG, P>0.05). The other biochemical parameters evaluated were not significantly affected by nitrates' consumption (P>0.05) in either experiment.

Conclusions: The prolonged consumption of water with increased nitrate levels increase BUN and BHBA concentrations and, to some degree, impairs protein metabolism and glucose utilization. The dietary administration of clinoptilolite can ameliorate nitrates' effects on BUN, protein metabolism and glucose utilization, but not on BHBA concentration.

Nutrition & Metabolic Diseases

P01-001-033

Prevalence of subclinical hypocalcemia in German dairy herds

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Objectives: Cows with subclinical hypocalcemia have no clinical signs of hypocalcemia but are more susceptible to other diseases. The objective of this study was to determine the prevalence of subclinical hypocalcemia in German dairy herds.

Materials and Methods: A multi-center study was performed involving 108 dairy herds and 26 different veterinary practitioners to determine the prevalence of subclinical hypocalcemia on German dairy farms. A sample size of about 1,300 animals with 12 animals per herd was required to estimate the true prevalence of subclinical hypocalcemia with 95 % confidence and 5 % precision. At least 12 serum blood samples were drawn on each farm from animals 0 to 48 hours after parturition. Calcium, magnesium and phosphorous was analyzed in a commercial laboratory. Subclinical hypocalcemia was defined as blood calcium below 2.0 mmol/l. Herds were classified into negative (0 to 2/ 12), borderline (3 to 5/ 12), and positive (≥ 6/ 12) according to the number of animals with subclinical hypocalcemia.

Results: So far, 348 samples from 29 herds have been analyzed. Overall, the prevalence of subclinical hypocalcemia in dairy cows was 36.8%. Prevalence increased with age and was present in 7.9%, 23.3%, 36.8%, and 55.1% of 1st, 2nd, 3rd, and ≥4th lactation animals, respectively. There were 5, 15, and 9 herds classified into negative, borderline, and positive for subclinical hypocalcemia, respectively. The number of animals with subclinical hypocalcemia in a herd sample ranged from 1 to 9 out of 12 (8.3 to 75.0%).

Conclusions: Preliminary results indicate that the prevalence of subclinical hypocalcemia in German dairy herds is high. Subclinical hypocalcemia is considered a gateway disease in transition dairy cows leading to other clinical diseases such as metritis and displaced abomasum. Therefore further research is warranted to estimate the effect of subclinical hypocalcemia on health, milk production and reproductive performance on a cow and a herd level.

Nutrition & Metabolic Diseases

P01-001-034

New mineral -vitamin blocks for dairy cows ration integrating utilizing domestic raw materials

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Objectives: Animal nutrition is often faced with scarcity of mineral substances in the feed ration, causing metabolic disorders and significantly affecting the animal's health and productivity. The objectives of the study was to investigate the new mineral substances and vitamin blocks for dairy cow ration integrating utilizing domestic raw materials.

Materials and Methods: For the research Latvian Brown cows were selected and assigned by the analogy principle to two treatments groups (n=2x12). Lactation dairy cows were included in the trial in the initial lactation phase with the average milk yield of 20.33 kg per day, fat content 4.03% and 3.64% protein content in milk. During the experiment the total mix ration (TMR) contained: 20 kg (36.12%), maize silage, 16 kg (45.15%) cereal grass- clover silage, 2 kg (4.51%) hay (cereal grass-clover), 4 kg (9.03%) concentrated feed mixture (rape oil-cake-barley meal), 2 kg (4.53%) protein concentrate, 0.1kg (0.22%) mineral premix ViloMin 9/6, 0.1 kg (0.22%) sodium chloride and 0.1 kg (0.22%) sodium carbonate. According to the investigation method, the control group of cows was fed traditionally complete feed rations, but experimental group in the feed ration were included for each cow individually mineral and vitamins complementary blocks. The forage analyses were made in the accredited laboratory of chemical analyses of the Latvia University of Agriculture, according to the EU ISO 6496 standard. Milk analyses were made of a/s "Siguldas CMAS" the milk control laboratory using Milkosan equipment according to ISO 9622:1997 standard. The data mathematical processing was made applying the program MS Excel. Credibility of results was tested by t-Test.

Results: Productivity indicators analysis showed, that within 151 days of study the experimental group had a milk yield of 19.1 kg ECM per day higher than in the control group. During the research, milk composition by its fat and protein content in the experimental group was increased by 0.16% and 0.12% compared with the control group (p <0.05). The research demonstrated the favorable influence of complementary mineral-vitamin blocks on the decrease of somatic cell count in milk: somatic cell count decreased by 202.07 thsd. ml⁻¹ in the experimental group and 250.77 thsd. ml⁻¹ in the control group cows. The economic effectiveness of feeding out the mineral-vitamin block was positive. Each experimental group of cows, which was fed on the complementary mineral-vitamin blocks gave on 2.94 euro higher milk output than their counterparts in the control group.

Nutrition & Metabolic Diseases

P01-001-035

Feeding, growing and finishing Friesian calves with diets supplemented with soybean oil and vitamin E

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Objectives: In recent years it has been held a lot of research on the manipulation of the fatty acid composition of intramuscular fat of beef through the supplementation of diets with polyunsaturated oils. However, little is known about the effects of such supplementation on sensory quality of beef, and it is possible that the greater susceptibility to oxidation of the more unsaturated intramuscular fat may result in changes in color and organoleptic properties.

Materials and Methods: In this work we tested the effect of supplementing feed for finishing calves with soybean oil and vitamin E on growth parameters and sensory quality of meat. We used 63 Friesian castrated male calves with 6 months of age, randomly assigned to three treatments (a pen per treatment with three replicates) fed with compound feed and straw ad libitum for 60 days before slaughter. The compound feed included a supplement of 4% saponified palm oil (T1), 4% soybean oil (T2) and soybean oil 50mg/kg + Vitamin E (T3). Experimental feeds were formulated to be isoenergetic and isonitrogenous. During this period, the animals always had free access to good-quality water and straw.

Results: The animals of treatment T1 and T2 had a lower feed efficiency than animals from T3 (5.62 and 5.47 vs 5.22 kg of feed /kg BW, respectively), the same effect was observed in ADG. The dietary treatments had a significant effect on meat characteristics. Meat from animals of T2 had a significantly higher pH value, whereas T3 produced a meat with values significantly higher than the other treatments in both the L* value (37.9 vs. 34.5 and 35.2 for T3, T1 and T2, respectively) and the b* value (3.83 vs. 2.53 and 2.41 for T3, T1 and T2 respectively). However, supplementation with soybean oil and vitamin E did not affect the shear force value of meat there was a huge difference in shear force due to ageing. The meat of animals T3 had a significant higher amount of fatty acids C20:5 n-3 EPA (1,4 vs 1,2 and 1,0 mg/100g in T3, T2 and T1 respectively) and more C22:5 n-3 (6,2 vs 5,8 and 5,4 mg/100g in T3, T2 and T1 respectively) which result in a significant difference in the content of n-3 fatty acids (11,7 vs 14,8 and 16,2 for T1, T2 and T3 (P=0,0048)). The evaluation by the tasting panel showed no preference, only a very high global acceptance of meat of all treatments.

Conclusions: Our results suggest that supplementation of diets for growing / finishing calves with 4% soybean oil allows for higher feed efficiency and that supplementation with soybean oil + Vitamin E 50 mg/kg also allows for a meat with a lighter color, usually appreciated by consumers, without affecting its tenderness.

Nutrition & Metabolic Diseases

P01-001-036

The effect of injectable trace mineral supplementation on the semen quality of bulls.

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Objectives: Reproductive performance of cattle is the main profit driver of most cattle farms around the world. While reproduction may be influenced by several factors, correct mineral balance is crucial to the reproductive performance of all species, including cattle and sheep. The aim of this study was to assess the impact of a commercially available

trace mineral injection containing 40 mg/mL Zn, 15 mg/mL Cu, 10 mg/mL Mn and 5 mg/mL Se (Multimin Cattle, Virbac SA) on the sperm and semen characteristics of high genetic merit Brahman and Simmentaler bulls managed by the Faculty of veterinary Medicine, University of Pretoria (South Africa).

Materials and Methods: Experimental animals consisted of 5 Brahman and 5 Simmentaler 4-6 year old confirmed bulls with recorded data on spermatogenic function. Animals were purchased from commercial operations and then brought to the experimental site. All animals were in good condition and free of reproductive and other diseases including trichomoniasis, vibriosis, brucellosis, bovine tuberculosis, EBL and BVD (PI). Animals were fed a balanced ration which was supplemented in trace minerals with a commercial mineral mix prior to, and throughout the study period. Body condition scores (BCS) and body weight ranged from 3.5-4 and 420-750 kg, respectively. On day D0, animals were randomly allocated to either group M (treated) or group C (Control) and each group consisted of 2 Brahman and 3 Simmentaler bulls. Group M animals were dosed subcutaneously with the trace mineral injection at a rate of 1 mL/50 kg while group C animals received a sterile saline injection at the same dose rate. Animals were followed for 3 months after the initial injection. Semen was collected twice a week and immediately analysed to assess its quality (volume and aspect, spermatozoa (SPZ) activity, SPZ defects). Mean concentration of spermatozoa (SPZ), total volume of semen collected over the study period, total of SPZ collected, mass motility score (MMS) and SPZ individual motility, percentage of motile SPZ and percentage of SPZ with no morphological defects were reported. The ejaculate was the statistical unit and threshold of statistical significance was set to 0.05.

Results: Animals with poor MMS (<2) or a very low proportion of motile SPZ (<70%) would be excluded from commercial artificial insemination operations. Thus one animal was withdrawn from group C (all ejaculates MMS=0, except one) and one animal was excluded from group M (motile SPZ ranging 0-39%, all time points). Spermatozoa concentration was numerically higher (t-test, P=0.077) in Group M than in Group C. However, the proportion of ejaculates with more than 1.2x10⁹ SPZ/mL was significantly higher (Chi-2, P=0.038) in group M. Proportions of ejaculates with more than 2.0x10⁹ SPZ was also numerically higher in treated animals (Chi-2, P=0.095) than in untreated controls. Total volume of semen collected over time did not differ between groups but total SPZ collected during the study period was numerically higher in Group M than in group C. Between groups, the MMS did not differ (range 0-5, cut-off: 2, Chi-2). Proportions of ejaculates with more than 70% of motile SPZ also did not differ (Chi-2, P=0.417). Proportions of ejaculates with more than 70% of morphologically normal SPZ did not differ but the proportion of ejaculates with more than 10% of SPZ with nuclear defects was significantly lower (Chi-2, P<0.01) in group M animals than in group C.

Conclusions: Injection of trace minerals in advance of the semen collection process increases the proportion of ejaculates with a high concentration of SPZ. A positive trend was also observed for total SPZ collected over the study period (which is of importance for the production of artificial insemination straws). No differences have been reported in MMS and SPZ motility. Injection seemed to have a positive effect on the SPZ nucleus but impact of the treatment on SPZ physiology requires further investigation.

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Nutrition & Metabolic Diseases

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Outbreak Of Polioencephalomalacia In A Sheep Flock In Northeastern Brazil

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Objectives: Polioencephalomalacia (PEM) is a neurologic disorder of ruminants characterized by necrosis of the cerebral cortex. Clinical signs include blindness, ataxia, and sometimes recumbency with seizures. The present work aimed to report the clinical, laboratorial, and pathological findings of an outbreak of PEM in sheep from Rio Grande do Norte state, Northeastern Brazil.

Materials and Methods: During anamnesis the rancher stated that seven days previously seven sheep showed ataxia, stiff gait, and blindness evolving to lateral recumbency in approximately 5-7 days. The sheep were raised in a confinement system with access only to triturated soybean shells in the past month. Two sheep already died and two severely affected sheep were presented to the Veterinary Hospital. Clinical examination, hematological profile and cerebrospinal fluid analysis were performed. During hospitalization, the two remaining sheep died and all four sheep were necropsied.

Results: Clinical examination revealed lateral recumbency with pedaling, dehydration, grinding of the teeth, nystagmus, blindness, dilated pupils, tachycardia, and ruminal hypomotility. This two sheep and four other animals with initial signs were treated with thiamine and dexamethasone; four with initial signs recovered and the other two died. Hematology and cerebrospinal fluid analysis showed no alterations for the specie. Moderate cerebral edema was observed at necropsy. The main histopathological changes were in the cortex of occipital, temporal, parietal and frontal telencephalic lobes. Microscopic cortical lesions included segmentar laminar necrosis, spongiosis, swollen of vascular endothelial nuclei, nuclear picnosis, and mild infiltrate by neutrophils and eosinophils. In this flock, PEM was caused by the prolonged ingestion of soybean shells as the only food available. This dietary management increased the population of thiaminolytic microorganisms. Thiaminase can be the result of bacterial production that is associated with high-concentrate feeds, and it can be found in certain plants or secondary to drug usage.

Conclusions: The authors reiterate the necessity of more awareness by the ranchers in providing good quality roughage for livestock, and thus minimizing the frequency of diseases related to high-concentrate feeds.

Nutrition & Metabolic Diseases

P01-001-038

The Biogenic Amine Gaba In Grass Silages Part 2 The Influence On Dairy Herd Health And Performance. Clinical Case Documentation

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Objectives: Feeding cattle grass is a high quality basic raw material. During ensiling, a marginal to strong true protein breakdown can occur mainly during the first 24 - 48 hours after covering the silo with the plastic (Muck and Pitt, 1993). Through bad fermentation conditions the formation of not desired products, e.g. GABA, is preprogrammed. In the following clinical case documentation, the influence of a GABA intake with the grass silage of approx. 100 g /cow/day over a period of 120 days and the consequences for health and performance of the dairy herd are described.

Materials and Methods: Since approx. 1996, an unspecific disease with severe problems, high incidence of ill cows and total losses, was observed in several dairy farms in Lower Saxony near the German North Sea coast. This disease was independent of good or bad farm management. As an example, the typical situation of one of these farms is described as follows:

- 1.) The dairy farm was well managed
- 2.) The cattle were housed in modern barns
- 3.) Whole cattle number: 120 dairy cows, nearly 120 heifers and calves
- 4.) Milk yield: 7928 kg, milk fat: 339 kg (4.28%), milk protein: 265 kg (3.34%)
- 5.) Initially good herd health
- 6.) The ration for the dairy cows (day 1- 120) consisted of
 - 6.1) Grass silage (first cut), obviously in good quality approx. 10 kg DM/cow/day (laboratory test results see below)
 - 6.2) Maize silage approx. 3 kg DM/cow/day
 - 6.3) Concentrated feed approx. 6-8 kg/cow/day (corresponding to the milk yield)
 - 6.4) Special concentrated feed 2 kg/cow/day
 - 6.5) Mineral mix 0.2 kg/cow/day

Laboratory test of the first cut grass silage: dry matter g/kg – 361, crude protein (cp) g/kg - 213, true protein (tp) g/kg – 79 (tp in % of cp 37,0), pH – 5,7: "result good". As the GABA concentration in the grass silage first cut seemed to be very high, we did 4 analysis (Evonik Nutrition & Care, Hanau) showing 10 g/kg DM on average. The resulting intake of GABA was approx. 100 g cow/day.

Results: Since mid of October (day 70-75 after starting the grass silage intake), the herd health was reduced remarkably and changed for the worse with the following clinical signs:

- | | |
|--|---------------------------------|
| 1.) Behavior: | weary |
| 2.) Movement | painful and slow |
| 3.) Going into the milking parlour | unwillingly |
| 4.) Milk yield: | reduced |
| 5.) Feed intake: | reduced |
| 6.) Weight: | loss |
| 7.) Manure: | dark-green, creamy-watery |
| 8.) Downer cow syndrome: (after parturition) | increased (ten to twelve weeks) |
| 9.) Displacement of the abomasum: | increased |
| 10.) Placenta retention: | increased |
| 11.) Mastitis and high SCC: | increased |
| 12.) Aggravation of "simple" diseases | |
| 13.) Treatment of diseases: | with lower success |
| 14.) Loss of cows: | increased |
| 15.) Sudden death: | several cows |

At day 120 (mid of December) after starting feeding the first cut grass silage (mid of August), the ration was changed to a second cut grass silage with GABA concentrations of 3.2 g GABA/kg DM (average n= 4). So, the total GABA intake was reduced from 100 g/cow/day (first cut) to

32 g/cow/day (second cut). Approx. 4-6 weeks after the change of the grass silage in the ration the dairy herd appeared completely changed with markedly increased health improvement. Only the critically sick fallen cows had no chance to recover.

Conclusions: Decreased true protein and increased GABA concentrations could be observed in Northern Germany during the last 20 years. In our case documentation, there was an intake of nearly 100 g GABA/cow/day for 120 days. Based on our experience over years, we can postulate that the intake of 40-45 g GABA/cow/day will be tolerated. But there is an enormous risk for disease in high yielding cows if the total GABA intake exceeds >45 g/day for a longer period (at least > 8 weeks).

Nutrition & Metabolic Diseases

P01-001-040

The effect of addition anionic salt in prepartum period in cows on chemiluminescence of PMN cells.

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Objectives: It is known, that the periparturient period is characterized by immunosuppression. In state of acid-base imbalance there is also observed a decrease of immunity.

The purpose of added anionic salt to the ration of dry cows before calving is to achieve a state of compensated metabolic acidosis. The aim of this study has been to evaluate the impact of addition anionic salt on PMN cells chemiluminescence (CL) level in last three weeks before parturition.

Materials and Methods: The study was conducted on 20 Polish HF cows before the third lactation. All the cows received the same TMR but 10 of them (experimental group) got extra 100g anionic salt ($MgSO_4 \cdot 7H_2O$) / cow/ a day for three weeks before parturition.

The blood samples were taken from their tail veins in -3, -2 and -1 week precalving. At the same time there were collected urine (by catheter) for urinary pH determination. The samples were collected into tubes containing K_2EDTA and lithium heparin. In whole blood there were determined level of chemiluminescence and haematological indices (BioOrbit 1251 Luminometer; ABCVet, Horiba ABX, France). Blood gas and pH were also analysed (Analyser 248 Ciba-Corning).

Results: Before parturition anionic salt supplemented cows urine pH was lower than control cows. Also blood pH the experimental group was lower in comparison with the control group. Oxidative metabolism of PMN cells, expressed as the level of chemiluminescence, was similar in the two groups. The level of chemiluminescence before parturition was determined by function of time. The main way to induce phagocytic activity was receptor pathway – CL induced by opsonized zymosan. There was no CL response to extrareceptor stimuli.

Conclusions: The addition of the anionic salt had no effect on the level of spontaneous and induced chemiluminescence. There was observed a tendency to decrease the level of CL but the differences were not statistically crucial.

Nutrition & Metabolic Diseases

P01-001-041

Effects of monensin and essential oils on fat mobilization and ketosis in dairy cows in the transition period

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Objectives: Ketosis as a consequence of negative energy balance in early lactation is a frequent health disorder in dairy cows. In particular over-conditioned cows are at risk of ketosis. The ionophore antimicrobial agent monensin alters ruminal flora in favor of propionate producing microbes and is approved for prevention of ketosis. Recently it has been reported that essential oils distilled from plant material have similar effects and may provide an alternative to monensin. The aim of this study was to compare effects of monensin and essential oils on fat mobilization and ketosis in dairy cows during the transition period.

Materials and Methods: The study was performed at the Friedrich-Loeffler-Institute (FLI), Braunschweig, Germany. 60 German Holstein cows were allocated at first to two groups according to their body condition (BCS). Cows with low BCS (LC; n=15, mean BCS 2.77 ± 0.14 (\pm SD)) were used as positive control and received diets with 20% concentrate ante partum (a.p.). Cows with high BCS (n=45, mean BCS 3.95 ± 0.08 (\pm SD)) were subdivided to form the negative control (HC/C, n=15), or to receive either a monensin controlled-release capsule (HC/MO, n=15) or a blend of essential oils (HC/EO, n=15) and were fed diets with 60% concentrate proportion a.p. Postpartum (p.p.) all groups were initially supplied with 30% concentrate, which constantly was increased to 50%. For feeding automated weighing troughs were used. Cows were studied from d-42 a.p. until d56 p.p.. Blood samples were collected in weekly intervals from all cows (analysis of glucose, beta-hydroxybutyrate (BHB), fatty acids (NEFA)) and sonography was performed to assess subcutaneous and total abdominal fat mass in a subgroup of eight cows of each group on d-21, d7, d21 and d56 relative to parturition. Also dry matter intake (DMI), milk yield, milk constituents and energy balance were recorded. Results were statistically evaluated in a mixed model with group, time and interactions as fixed effects and cow as random effect with Tukey test for multiple comparisons of means (SAS package 9.3).

Results: Mean DMI was lowest before and highest after parturition in the LC- compared to HC-groups (group*time: $p < 0.001$). Treatment had no significant effects on milk yield and 4%fat corrected milk yield. In average energy balance was significantly higher in HC-groups than in the LC group before parturition, which was reverse after parturition (group*time: $p < 0.001$). After parturition mean blood NEFA were significantly lower (group*time: $p = 0.013$) and BHB concentrations higher in the LC group than in HC-groups. However, from HC cows those of the HC/MO-group presented mean BHB levels lower than of cows of HC/C and HC/EO and numerically lower than of cows of the LC-group (group*time $p = 0.008$). Loss of subcutaneous and total abdominal fat mass assessed in kg/day after parturition revealed no treatment effects.

Conclusions: Mean blood BHB level was significantly reduced in monensin treated cows although monensin revealed no effect on lipomobilization after parturition according to blood NEFA concentrations and daily adipose tissue weight changes. Results suggest that a monensin controlled-release capsule could promote a more effective hepatic or extra hepatic NEFA or extra hepatic BHB utilization in dairy cows after parturition, thus reducing the risk of subclinical ketosis, whereas essential oils failed to produce a comparable effect.

Nutrition & Metabolic Diseases

P01-001-042

Association Of Virginiamycin And Monensin Mitigates The Rumen Lactic Acidosis Effects In Cattle

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Objectives: Rumen lactic acidosis (RLA) is a common disease in feedlot cattle. For prevention of this illness some dietary ionophores and antibiotics are used, mainly monensin (M) and virginiamycin (V). This experiment was carried out to check, by the first time, the effects of preventive use of dietary V + M on an experimental RLA in cattle.

Materials and Methods: An Official Brazilian Animal Ethics Commission approved this protocol, previously. Twelve yearling Nellore heifers were rumen cannulated and fed for one month with a 75% roughage and 25% concentrate diet according to their 2.5% body weight. Six heifers received daily 250 mg V and 300 mg M (V+M) in the diet and the same number kept as a control (C). An experimental RLA was induced in all animals by infusion of different amount of sucrose, into the rumen cannula, according to a formula based on a corrected metabolic weight. For the next 18 h some rumen parameters (pH; L- Lactate level; titratable acidity and redox potential), hematological (plasma volume deficit PVD) and clinical picture were followed. For complete recovering many animals needed, at the 18h, a treatment based on withdraw of all rumen fluid and replacement with healthy rumen fluid and water, and intravenously infusion of large amount of Lactated Ringer Solution. The distribution of all data was evaluated using the Kolgomorov-Smirnov test followed by repeated measures modelling with PROC-MIXED and coefficient of determination (R²) in the SAS.

Results: Rumen pH and L-Lactate levels were lower and higher, respectively, at the 3rd, 6th and 18th h in the control group (P= 0.019). Both titratable acidity (12th and 18th h), and redox potential (3rd, 6h, and 12th) were higher in the control group (P = 0.03). The R² for rumen pH with the following variables L-Lactate level, redox potential and titratable acidity were 0.91, 0.96 and 0.87, respectively. These results suggested that the principal acid produced in this induction was L-Lactate and somehow V+M decreased the production of this acid probably by interference with Lactobacillus sp population growth, most important rumen lactate producer, since this bacteria requires very high rumen redox potential to growth. There was a tendency (P= 0.1) for the PVD to be higher in the control group at the 18th h, suggesting a more developed degree of dehydration. Most of the cattle (4/6) of the control group exhibited pronounced clinical signs as compared to the V+M group (1/6), mainly shown by severe dehydration (sunken eyes and coldness in the extremities), recumbency, mental depression that required rapid treatment for complete recovery thereafter.

Conclusions: This experiment showed that V+M used preventively could mitigate the risk of a very severe RLA by decreasing the production of rumen lactate, and the rumen redox potential and thus avoiding a very sharp drop in the rumen pH that could cause harmful clinical signs.

Nutrition & Metabolic Diseases

P01-001-043

Effect of duration and temperature during the delivery of bovine urine samples to the analytical laboratory on metabolic parameters assays

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Objectives: Urine samples collected in bovine medicine are often exposed to a range of environmental conditions prior to analysis in a laboratory. The stability of the molecules in the bovine urine is poorly understood. The aim of this study was to determine the stability of urine specimens exposed to a range times and two storage temperatures.

Materials and Methods: Urine samples were taken with a urinary catheter in 10 lactating Montbeliarde cows in one farm, 1.5 hours after the morning diet distribution. The samples were immediately placed in a cooler box, protected from light, and sent to the laboratory. For each cow, the sample is divided into 17 aliquots. one aliquote was centrifuged and analyzed in the 3 hours after collection (D0) as controls. 8 aliquots, were placed in a refrigerated chamber at 4°C and 8 aliquots, left at room temperature (25°C). All the samples were kept sheltered from the light. The day D1, D2, D3, D4, D7, D8, D9, D10, one aliquote stored at room temperature and one kept cool tube were centrifuged and analyzed. The analysis method used was a enzymatic colorimetric method with an automated biochemistry analyser (JEOL Biomajesty 6010 with Biomérieux, Diasys, Diacron and Randox kits).

Statistical analysis was performed with the Excel © software. For each biochemical parameter, outliers were removed by the Grubbs test. For each pair number storage day / storage temperature and each parameter, the averages of the 10 cows was compared with the average to D0 by a Student's t test for paired data if normal distribution (checked by Shapiro-Wilk test) and variance equal (verified by Cochran's test) or by test of Wilcoxon signed rank in other cases. The results of stored samples were considered to be different from the value on D0 (reference) if the difference between average of stored samples and D0 average was greater than the measurement uncertainty or if p> 0.05.

Results: Assay results are identical to the D0 value during the numbers of days (d) following : (for samples stored at 4°C and 25°C respectively) ; calcium 10 d, 10 d ; chloride 10 d, 10 d ; total carbon dioxide 7 d, 7 d ; creatinine 10 d, 10 d ; magnesium 4 d, 4 d ; Phosphate Inorganic 2 d, 2 d ; Potassium 10 d, 10 d ; Sodium 10 d, 10 d ; Urea 10 d, 10 d.

Our results show a stability of creatinine much longer than that reported by Saliman et al in 1986, against we find the same duration stability for urea.

Conclusions: The storage temperature has no effect on the duration of biochemical parameters stability in urine until 10 days.

The stability of biochemical specimens is very long, it reached 10 days except for Phosphate Inorganic (2 d.) and to a lesser extent magnesium (4 d.).

The duration stability in urine is better than in blood except for Phosphate Inorganic and magnesium. These results show a very good stability of urine parameters in cattle. The use of this biochemical assays should not be limited even if the delivery times in the laboratory is several days.

Comments: Soliman S.A., Abdel-Hay M.H., Sulaiman M.I. Tayed O.S. 1986. Stability of cratinine, urea and uric acid in urine stored under various conditions. Clinica Chimica Acta, 160 319-326.

Nutrition & Metabolic Diseases

P01-001-044

Injectable Mineral supplementation of phosphorus and selenium in addition to oral free-choice mineral mixes in young Nelore cattle in Brazil.

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Objectives: In Brazilian beef cattle operations, delivery of free-choice mineral mixes is the most common way of supplementing animals with minerals. However, in grazing animals, free-choice oral supplementation poses several problems which reduce mineral bio-availability, meaning that animals often do not get adequate amounts to meet their minimal requirements, as recommended by the NRC. The aim of this study was to determine the effect of a single injection of sodium glycerophosphate (1.40 g/10mL), sodium phosphate (2.01 g/10mL) and sodium selenite (0.024 g/10mL) (P-Se) (FOSFOSAL®, Virbac Saúde Animal, Brazil) in fast growing, pasture raised cattle.

Materials and Methods: Four distinct experiments were performed in three Brazilian beef operations located in Itapetininga (São Paulo), Santa Rita do Pardo (Mato Grosso do Sul) and Caceres (Mato Grosso). Experimental animals consisted of 256 mixed sex Nelore breed cattle. Three experimental groups consisted of all male animals, while one experimental group contained equal distribution of both sexes. The average age of animals ranged from 7 to 12 months within groups. Across all operations, animals grazed predominantly *Brachiaria decumbens* pastures with ad libitum access to free-choice oral mineral supplementation, in accordance with the normal management practices of the farms. In each experiment, animals were randomly allocated into two groups of equal size and treated according to body weight with a single subcutaneous injection of either a single injection of P-Se (group F) or sterile saline (group C) on Day 0. Both groups of animals were maintained on the same pasture under the same conditions and dietary changes during the study period. All animals also received a routine antiparasitic treatment (ivermectin) at the beginning of the study. The study period lasted approximately one month. Animals were weighed on Day 0 and again on the last day of the study. Individual Average Daily Gains (ADGs) were calculated in kg/day. Statistical analysis (t-test) was performed on group ADG using BioSta TGV.

Results: Animals were weighed between days 29 to 38. In Itapetininga(1), Itapetininga(2), Santa Rita do Pardo and Caceres reported ADGs(±sd) were 0.356(±0.173) vs. 0.467(±0.186), 0.054(±0.169) vs. 0.302(±0.180), 0.446(±0.294) vs. 0.605(±0.256) and -0.058(±0.409) vs. 0.046(±0.193) for Groups C and Groups F, respectively. In all but one experiment, differences were significant (p<0.05). Further investigations are needed to confirm these results for longer periods in this class of stock and also to determine if other stock classes (heifers, cows and bulls) could benefit from a similar approach.

Conclusions: While it is essential to have an efficient oral mineral supplementation program in place to avoid clinical deficiency, at the herd level, there may be some benefit in supplying minerals via an alternate route, namely via injection at strategic times of the production cycle. Under standard Brazilian grazing conditions, providing injectable P-Se to young, growing Nelore cattle in addition to a free-choice oral mineral supplement looks very beneficial, resulting in higher ADGs in treated animals compared to controls animals. In 3 experiments out of 4, differences in mean ADGs between treated and untreated animals were significantly different.

Comments: Authors greatly acknowledge staffs of the farms.

Nutrition & Metabolic Diseases

P01-001-045

Effect Of An Injectable Supplement Containing Vitamin E And Selenium Pre-Calving On The Postpartum Response To Glucose Tolerance Test In Dairy Cows

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Objectives: Dairy cows undergo a period of insulin resistance (IR) following calving, but the mechanisms causing IR are not fully understood in cattle. In human type II diabetes, there is strong evidence that oxidative stress (OS) plays a causative role in the development of IR, and antioxidant supplementation can be used to reduce its consequences. Transition dairy cattle experience OS, and a significant association between OS and whole-body insulin sensitivity has been recently reported during this period. Hence, this trial aims at establishing whether antioxidant therapy enhances the response to glucose tolerance tests in early lactating cattle.

Materials and Methods: Ten close-up dry cows from the same herd were randomly allocated to one of the study groups. Two weeks prior to expected calving, the treatment group received an IM injection (Selevit adultos, Laboratorios SYVA, Leon, Spain) containing 6 mg/kg of DL-tocopherol acetate (vitamin E) and 0.06 mg/kg of sodium selenite, whereas the control group was sham injected with isotonic saline. Three to 7 days after calving cows in both groups underwent a glucose tolerance test (GTT). For the analytes glucose, insulin, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) the following parameters were calculated and compared between groups with the Mann-Whitney U test: areas under the curve (AUC), peak and nadir concentrations and elimination rates.

Results: No differences between groups were found for the concentrations of the metabolic markers studied either at the time of group allocation or at the basal GTTs measurements. With regards to the response to the GTTs, differences between groups were found for the AUC for glucose (P=0.009), insulin (P=0.043) and NEFA (P=0.041), along with the elimination rates of NEFA (P=0.047) and insulin (P=0.008). A trend to significance was found for the glucose elimination rate (P=0.076). These results showed an improved energetic metabolic response to GTTs in the animals supplemented with vitamin E and Selenium pre-calving, with an increased whole-body insulin sensitivity. Thereby implying that oxidative stress plays a causative role in post-parturient insulin resistance in dairy cattle and that antioxidant therapy might be beneficial for ameliorating the harmful consequences of a prolonged IR state.

Conclusions: Parenteral supplementation with vitamin E and selenium two weeks before calving improved the animals' response to glucose tolerance tests during the first week after calving, showing higher insulin sensitivity. Hence, it is concluded that oxidative stress plays a causative role in the development of IR in dairy cattle, and that antioxidant therapy can ameliorate the detrimental effects of prolonged IR.

Nutrition & Metabolic Diseases

P01-001-046

A meta-analysis of lasalocid effects on rumen measures, beef and dairy performance, and carcass traits in cattle

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Objectives: The objective of this study was to evaluate the effects of lasalocid feeding on rumen measures, beef and dairy performance, and carcass traits in cattle, using meta-analytic methods to describe its impact and explain heterogeneity of responses among studies.

Materials and Methods: Literature searches were performed in PubMed, Google Scholar, and ISI Web of Knowledge using the following search: (Lasalocid OR Bovatec) AND (cattle OR cows OR feedlot OR calves OR dairy OR beef). Studies were included if they met the following criteria: were full manuscripts from peer-reviewed English language journals, evaluated use of lasalocid supplementation against a control, described randomization process, had a randomized controlled trial design only, animals studied were weaned cattle, they included replication of treatments, sufficient data to determine the effect size (ES), a measure of effect amendable to effect size analysis for continuous data (e.g. standardized mean difference, SMD); and a measure of variance (SE or SD) for each effect estimate or treatment and control comparisons. Only variables with a minimum of 6 comparisons were evaluated. Stata v. 11 (StataCorp. LP, College Station, TX) was used to analyze output variables by SMD and only random effects models were used. The difference between lasalocid treatment and control groups means was standardized using the SD of control and lasalocid treatment groups. The SMD estimates (ES and 95% CI) were pooled using both the DerSimonian and Laird and Knapp-Sidik-Jonkman methods for random effects models to evaluate the effect of study and displayed in forest plots. Heterogeneity was assessed using a chi-squared (Q) test of heterogeneity. Meta-regression analyses were used to explore the source of heterogeneity of response. The presence of publication bias was investigated using contour-enhanced funnel plots.

Results: Rumen measures were assessed using 10 studies (20 comparisons). Lasalocid increased total VFA and ammonia concentrations by 6.46 and 1.44 mM, respectively. Lasalocid increased propionate and decreased acetate and butyrate molar percentage (M%) by 4.62, 3.18, and 0.83%, respectively. Valerate M% and pH were not affected. Meta-regression found butyrate M% increased linearly with duration of lasalocid feeding (DUR; $P = 0.017$). When >200 mg/d was fed, propionate and valerate M% were higher and acetate M% was lower ($P=0.042$, 0.017 , and 0.005 , respectively). Beef performance was assessed using 31 studies (67 comparisons). Lasalocid increased ADG by 40 g/d, improved feed-to-gain (F:G) by 410 g/kg, and improved feed efficiency [FE; combined gain-to-feed (G:F) and inverse of F:G]. Lasalocid did not affect DMI. Heterogeneity in DMI was influenced by DUR ($P=0.004$) and linear effect of entry BW ($P=0.011$). Heterogeneity of ADG was influenced by the linear effect of entry BW ($P=0.028$). The FE ($P=0.025$) and F:G ($P=0.015$) improved linearly with dose, and entry BW >275 kg improved F:G ($P=0.038$). Carcass traits were assessed using 14 studies (25 comparisons). Lasalocid increased HCW by 4.73 kg, but not dressing percentage, mean fat cover, or marbling score. Heterogeneity of carcass traits was low and not affected by DUR or dose. Dairy performance was assessed using 7 studies (11 comparisons) but study power was relatively low. Lasalocid decreased DMI in TMR-fed cows by 0.89 kg/d, but had no effect on milk yield, milk components, or component yields. Dose linearly decreased DMI ($P=0.049$).

Conclusions: This work showed lasalocid improved ADG, HCW, FE, and F:G for beef production. These findings may reflect improved

energy efficiency from increased propionate and decreased acetate and butyrate M%. Large dairy studies are required for further evaluation of effects of lasalocid on dairy performance. These results allow producers, nutritionists, and veterinarians to make informed decisions on the inclusion of lasalocid in cattle diets.

Nutrition & Metabolic Diseases

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Impacts of a monensin capsule administered within thirty days of calving on health and milk production in lactating dairy cattle - a meta-analysis

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Objectives: The objective of this project was to conduct a meta-analysis of research on the health and production effects of administration of a monensin controlled release capsule within thirty days of calving to provide a summary of expected effects and heterogeneity of response.

Materials and Methods: A database containing data from papers identified with a previous meta-analysis (Duffield et al, 2008) were utilized as a starting point for the data acquisition. A total of three additional papers were identified for inclusion beyond what was previously reported. In addition, the data from the European registration trial for the monensin controlled release capsule (Kexxtone™) was acquired for inclusion. Reported research needed to involve monensin delivery via capsule within thirty days of calving, treatments must have been randomly assigned and compared to an appropriate control group. A mean, number of animals per group, and some measure of dispersion must have been included.

Data was extracted for several outcomes including cow health (including clinical ketosis, displaced abomasum, metritis, mastitis, retained placenta), and milk production (yield, fat percent, protein percent). A meta-analysis was conducted on the extracted outcomes using Stata (Intercooled Stata V. 13.1, USA). Effect size estimates were generated using a standardized mean difference for continuous outcomes and relative risk for dichotomous variables. Heterogeneity and percentage of variation across studies due to heterogeneity were both tested in each model with appropriate statistics. If there was evidence of heterogeneity, a random effects model was used.

Results: There were a total of nine papers, 14 trials, 105 trial sites and 5876 cows available to evaluate the impact of the monensin controlled release capsule on health outcomes. Similarly there were also nine papers, 18 trials, 98 trial sites and over 6000 cows to evaluate the impact of the monensin capsule on milk production parameters. Across all studies, milk production was increased with the administration of the monensin capsule two to four weeks prior to calving ($P=0.06$), however, there was significant heterogeneity in the data ($P=0.02$), indicating that the response varied by trial. The analysis using weighted mean difference did show a significant increase ($P < 0.05$) of an average of 0.6 kg/day of milk yield for the first 3 to 4 months of lactation. There was no effect of the capsule on either milk fat percentage ($P=0.36$) or milk protein percentage ($P=0.36$).

Analysis of health outcomes indicated that administration of the controlled release capsule prior to calving significantly reduced the incidence of both Clinical Ketosis (RR=0.70, $P < 0.001$) and Displaced Abomasum

(RR=0.80, P<0.001). The impact of monensin on retained placenta was not significant (P=0.16) but did have significant heterogeneity (P=0.02). There were no other significant findings for any of the other health outcomes.

Conclusions: In addition to the label indication of reduced incidence of subclinical ketosis, use of a monensin capsule prior to calving can be expected to reduce the incidence of clinical ketosis and displaced abomasum. In addition, an average of 0.6 kg/day increase in early lactation milk yield is expected, although this response varies between studies. The heterogeneity of response in milk yield is consistent with milk yield being associated with restoring milk production potential in high risk ketotic cows/herds. These benefits were found when administering prevention to all cows, thus responses would be expected to be better in high-risk cows.

Nutrition & Metabolic Diseases

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Carcass characteristics and meat evaluation of Nelore cattle submitted to different antioxidants treatments

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Objectives: This study aimed to evaluate the effects of supplementation with different natural antioxidants (zinc, selenium, vitamin E and selenium + vitamin E) on performance, carcass characteristics and meat quality of feedlot cattle fed with *Brachiaria* hay.

Materials and Methods: Forty Nelore cattle aged 24-36 months were used. Animals were fed *Brachiaria brizantha* hay and subjected to five treatments (control and four antioxidants: zinc, selenium, vitamin E and selenium + vitamin E). There were eight animals per group, each animal considered a replication. After 105 days feeding period, cattle were slaughtered at a commercial abattoir. After 24 hours in a cold room, the right halves of the carcasses were used to obtain pH values. The HH section was extracted between the 11th and 13th rib of the left half of each carcass, for determination of muscle, bone and fat percentage. Longissimus dorsi muscle samples, previously minced and homogenized, were used for determination of dry matter, ash and crude protein and fat content. The determination of the meat color was performed by CIEL*a*b* methodology using a colorimeter. Losses by thawing and in the cooking process, meat water retention capacity as well as shear force and lipid profile were evaluated in the Longissimus dorsi samples. Analysis of variance was carried out and the parameters with significant effect were compared by Tukey test, at 5% probability.

Results: There was variation in the carcass muscle percentage depending on antioxidant used. Selenium showed the lowest muscle percentage (66.61%) compared to the other antioxidants evaluated. However, there was no difference between treatments for bone, fat, comestible portion percentages and muscle: bone, muscle: fat and comestible portion: bone ratios with mean values of 16.85%, 14.70%, 82.99%, 4.06, 4.85 and 4.95, respectively. Neither brightness, red and yellow contents of the meat nor carcass pH were affected by treatments, with mean values of 29.29% of brightness and 6.31% of pH. For Longissimus dorsi muscle tenderness, losses on thawing and in the cooking process, there were no differences between treatments, with

averages 6.43 kg², 3.22% and 21.15%, respectively. There was variation in the fatty acid profile according to the antioxidant used. The fatty acids observed at the highest concentrations were myristic (C14:0, 14.15%), oleic (C18:1 n-9 cis /C18:1 n-9 trans, 13.73%), palmitoleic (C16:1, 13.47%), lauric (C12:0, 9.13%), eicosapentaenoic (C20:5 n-3, 8.91%), capric (C10:0, 8.43%) and palmitic acids (C16:0, 8.01%). Control, zinc and selenium treatments showed the highest levels of palmitic (C16:0) and stearic acids (C18:0) and lower levels of palmitoleic acid (C16:1). Higher amounts of palmitoleic (C16:1), heptadecenoico (C17:1), elaidic (C18:1 n-9 cis /C18:1 n-9 trans), linoleic (C18:2 n-6 cis/C18:2 n-6 trans) and DHA acids (C22:6 n-3/C24:1 n-9) and lower palmitic (C16:0) and stearic acids (C18:0) were observed for vitamin E and selenium + vitamin E treatments.

Conclusions: Supplementation of Nelore cattle fed *Brachiaria brizantha* hay with antioxidants did not influence carcass characteristics nor meat quality. However, vitamin E supplementation reduced the levels of omega 3, whereas supplementation with selenium + vitamin E promoted an increase in linoleic and palmitoleic acids, and reduced myristoleic acid, making the supplementation feasible due to the beneficial effects provided by these acids.

Nutrition & Metabolic Diseases

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Phenotypic correlations between subclinical and clinical postpartum health disorders in Holstein cows

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Objectives: The objective was to estimate the phenotypic correlations between serum Ca, P, Mg, K and β-hydroxybutyric acid (BHBA) concentrations, body condition score (BCS) and several postpartum health disorders during the first 8 days after calving in Holstein dairy cows.

Materials and Methods: The study included 1,021 Holstein dairy cows (1 - 4+ lactations) in 9 herds. No herd used preventive measures for hypocalcemia. Clinical examination and blood sampling was performed on DIM 1, 2, 4 and 8. Clinical cases of milk fever (MF), retained fetal membranes (RFM), metritis (MET), mastitis (MAST), left/right abomasal displacement (LDA/RDA), ketosis (KET), and uterine prolapse (UP) were recorded on the same days. Recordings of involuntary culling (INVCULL) and death (DE) were also performed on the same days. Serum concentrations of Ca and Mg were determined with AAS, while P and K with biochemical and electrolyte analyzers. BHBA was measured with a spectrophotometer in samples of day 8. The final data set included 4,020 Ca, 4,019 P, 4,020 Mg, 3,792 K, 997 BHBA measurements and 4,064 clinical records. Macromineral-related traits defined as: subclinical hypocalcemia (SCHCa): Ca_s≤8.3 mg/dL; hypophosphatemia (HypoP): P≤4.2 mg/dL; subclinical hypomagnesemia (SCHMg): Mg≤1.8 mg/dL; hypokalemia (HypoK): K≤3.9 mEq/L; and hyperphosphatemia (HyperP): P≥7.80 mg/dL. Relevant phenotypic correlations during the first 8 days after calving were estimated with bivariate analysis using random regression and single record models. The analysis for Ca, Mg, P and K and the macromineral-related traits included 986 cows, which showed no obvious signs of MF (35 cows with MF were excluded). The analysis for the other health traits included all 1,021 cows.

Results: Serum Ca had low negative phenotypic correlations with BHBA, RFM, MET, LDA and DE (r_p : -0.09 to -0.18; $P < 0.05$), moderate negative correlation with MF (r_p : -0.32±0.03; $P < 0.05$) and low positive correlation with BCS (r_p : 0.11±0.03; $P < 0.05$). Serum P had low positive correlations with MAST (r_p : 0.12±0.03; $P < 0.05$) and UP (r_p : 0.08±0.03; $P < 0.05$). Serum Mg had low negative phenotypic correlations with RFM, MET and LDA (r_p : -0.07 to -0.15; $P < 0.05$) and low positive correlation with BCS (r_p : 0.14±0.03; $P < 0.05$). Serum K had low negative phenotypic correlations with BHBA, MF, RFM, MET, MAST and DE (r_p : -0.09 to -0.14; $P < 0.05$). SCHCa had low positive correlations with BHBA, HypoP, SCHMg, HypoK, MF, MET and LDA (r_p : 0.07 to 0.15; $P < 0.05$). HypoP had low positive correlations with SCHMg (r_p : 0.07±0.03; $P < 0.05$) and low negative with HyperP (r_p : -0.09±0.03; $P < 0.05$). SCHMg had low positive correlations with BHBA, HypoP, HypoK, RFM, HyperP and MET (r_p : 0.07 to 0.13; $P < 0.05$). HypoK had low positive correlations with BHBA, MF, MET, RFM and LDA (r_p : 0.07 to 0.13; $P < 0.05$). HyperP had low negative correlation with BHBA (r_p : -0.07±0.03; $P < 0.05$) and low positive with MAST (r_p : 0.07±0.03; $P < 0.05$). BSC had low negative correlations with BHBA and SCHMg (r_p : -0.07±0.03; $P < 0.05$). MF had low positive correlations with RFM and LDA (r_p : 0.07 to 0.11; $P < 0.05$) and moderate positive correlations with UP and DE (r_p : 0.18 to 0.19; $P < 0.05$).

Conclusions: The estimated phenotypic correlations showed a strong association between macromineral serum concentrations, subclinical health disorders, clinical diseases and recordings immediately after calving, emphasizing the necessity for integrated preventive management measures, regarding nutrition and housing.

Nutrition & Metabolic Diseases

P01-001-050

Effect of Dietary Starch Levels In Early Lactation Dairy Cows

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Objectives: The objective of this trial was to determine the effect of dietary starch concentration on locomotion and body condition scores and performance of early lactation dairy cows fed diets of different starch concentrations.

Materials and Methods: This study was performed at the Research and Application Farm of the Faculty of Veterinary Medicine at Uludag University from September 2012 to May 2013. The Animal Experimentation Ethics Committee of Uludag University approved all the experimental procedures. Twenty-four primiparous Holstein cows were randomly assigned to 1 of 3 treatments in a completely randomized design from calving through 72 d DIM. Diets contained 30% corn silage, 20% chopped alfalfa hay, and 50% concentrate/mineral/vitamin/additive mixtures (on DM basis). The concentrate mixtures were formulated by partially replacing dry ground shelled corn with wheat bran. Cows were fed the low (LS), medium (MS) and high-starch (HS) diets as total mixed ration. The dietary starch contents were 16.2, 19.8, and 24.1% for low LS, MS, and HS, respectively (DM basis). Body condition score (BCS), body weight (BW) and locomotion scores were measured weekly throughout the trial. Dry matter intake and milk yield were recorded daily

on individual cows milked 3x. Data were analyzed according mixed models procedure.

Results: There was a tendency ($P = 0.07$) for locomotion scores to be greater for MS compared to LS. Milk yield averaged 29.4 kg/d and was unaffected ($P > 0.10$) by treatment. Values for milk urea nitrogen ranged from 12.4 to 13.0 mg/dL across the treatments and was lower for cows fed MS than for cows fed the LS. The BW, BCS, and milk fat and protein percentage measurements were unaffected ($P > 0.10$) by treatment. Dry matter intake for cows fed the LS diet was 1.1 and 2.3 kg/d greater than for cows fed the MS ($P < 0.05$) and HS ($P < 0.01$) diets, respectively.

Conclusions: It was concluded that when partially replacing corn grain with wheat bran to formulate reduced-starch diets, increased dry matter intake for dairy cows fed LS may reduce feed efficiency (kg of milk/kg of dry matter intake).

Nutrition & Metabolic Diseases

P01-001-051

Refusal to Drink in Calves in German Farms - Phenolic compounds a problem?

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Objectives: Since four years, veterinary practices throughout Germany reported problems in cattle populations of various production systems: Calves which had been drinking their colostrum normally on their first day of life quit drinking entirely within the following two days. Their vitality declined and finally they were recumbent. Even intensive clinical care including intravenous rehydration therapy was unsuccessful. Almost all affected calves died. Scientists of the Clinic for Cattle suspected intoxication with phenolic compounds from grasses (so-called secondary plant substances) in all cases.

Materials and Methods: Thus, blood samples were taken on the second day of life and analyzed for phenols, total protein and gamma-GT. Quantitative photometric analysis of free phenols in serum of these calves was performed. The free phenolic compounds were detected by using a sum reaction with Folin-Ciocalteu's reagent (method according to Holloway et al. 1979; modified by Flaschoff 1983) with the variation coefficient being less than five percent for this method. The currently used reference values of 0.26 to 0.42 mmol/l were provided by Hölting (1993).

Results: The determination of total protein in serum showed a good ingestion and absorption of colostrum: > 50 g/l total protein in the serum. In addition, gamma-GT levels in the blood of newborn calves were higher than 250 U/l indicate adequate colostrum quality. The scientists' suspicions were confirmed and increased phenol levels could be shown in affected calves (0.6 - 1.2 mmol/l). By early separating calves from dams - after having ingested sufficient amounts of colostrum - no later than two days after birth and by feeding milk replacer or milk from cows late in lactation, the disease progress could be stopped in all affected farms.

Conclusions: Phenols are compounds with one or several hydroxyl groups attached to a benzene ring. They occur in animals partly through breakdown of aromatic amino acids by intestinal bacteria. However, considerable amounts of phenol-containing compounds are also synthesized in plants. Their functions in a plant range from being pigments to acting as defence substances. Due to the abundance of different

molecules in this substance group, a more precise characterization of the molecules responsible for the refusal to drink in calves is currently not possible.

Nutrition & Metabolic Diseases

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Study of Liver Cobalt levels in Cattle and Sheep in Ireland

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Objectives: Cobalt deficiency has been shown to be a limiting factor in the performance of cattle and sheep production (Robertson 1971). A devastating syndrome in ruminants, now recognized in many parts of the world, was demonstrated in 1934 to be the result of cobalt deficiency, first in pastures of certain areas, then in harvested forages, and later in selected mixed rations. (Smith 1957). This study carries out analysis of cobalt levels in livers of cattle and sheep in Ireland with the aim to determine the required levels of cobalt in liver samples below which cobalt will not have a negative effect on the performance of cattle and sheep.

Materials and Methods: Recently weaned lambs (n=67) were allocated to one of the following 3 supplementation treatments: no supplement (control), Co (Cobalt) or Co, vitamin B12 plus Se (VitMin). The Cobalt treatment contained cobalt sulphate (2.1 mg/ml) only. The VitMin treatment contained B12 (200 µg/ml), cobalt acetate (10 mg/ml) and selenium selenite (0.25 mg/ml). Lambs on the Cobalt and VitMin treatments received 10 ml and 2.1 ml of drench, orally fortnightly until 18 November. Lambs were managed in a rotational grazing system on predominantly perennial ryegrass swards without concentrate supplementation. Lambs were slaughtered on either 6 November or 18 December. The day prior to slaughter a blood sample was removed from the jugular vein for determination of protein, urea, beta-hydroxybutyrate (BHB), Selenium (Se), Copper (Cu) and Glutathione peroxidase (GSHPX) concentrations. Following slaughter tissue was removed from the liver and the kidney cortex for determination of cobalt and selenium concentrations, respectively. The data were analysed as a randomised study using Proc MIXED of SAS.

Liver samples taken from cattle (n=70) and sheep (n=54) received for post mortem at Athlone Regional Veterinary Laboratory from 1st July 2015 to 30 Nov 2015 and the cobalt levels determined.

Results: The daily liveweight gain of the lambs supplemented with cobalt was 80% higher than those not supplemented from the 7th August until slaughter and 105% higher for lambs supplemented with B12+Co+Se. Relative to lambs supplemented with Co, those supplemented with B12+Co+Se had higher blood concentrations of selenium and GSHPX and higher liver Co concentration. The lambs slaughtered on the 6 Nov and 18 Dec received their last treatment drench 14 and 30 days prior to slaughter, respectively. Delaying slaughter date, and thus increasing the time interval from the last treatment drench, increased protein concentration and reduced BHB and urea concentrations in the blood, and reduced Co in the liver. Blood protein and the concentrations of Cu in blood and liver increased with delayed kill date. There was a

significant treatment x kill date interaction for Co concentration in liver. For the control, Cobalt and VitMin treatments liver Co concentrations were 0.11, 1.02 and 1.36 µmol/kg, and 0.23, 0.44 and 0.62 µmol/kg for kill date 5 Nov and 18 Dec, respectively.

In the study of cobalt levels in the livers of cattle and sheep received by Athlone Regional Veterinary Laboratory about 50% of liver samples from cattle are below 0.06ppm cobalt at which level cobalt is a limiting factor on performance (Marston 1949). 40% of liver samples from sheep are below 0.06ppm.

Conclusions: Supplementation with Co, alone or in combination with vitamin B12 and Se, increased liver Co concentration. The concentration of Co in liver declined by 55% following a 30-day period without supplementation.

The lower levels of liver cobalt in cattle versus sheep at Athlone Regional Veterinary Laboratory may reflect that sheep farmers are more likely to supplement with cobalt.

More work needs to be undertaken in Cattle on the effects of low liver cobalt/B12 on thrive in cattle in Ireland on pasture based systems.

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LIPOPEROXIDATION HEPATIC IN CATTLE FEEDLOT FED WITH BRACHIARIA SP HAY AND SUPPLEMENTED WITH ANTIOXIDANTS

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Objectives: Sporidesmin is an easily oxidized toxin produced by *Pithomyces chartarum*, a fungus that colonizes *Brachiaria* spp, and has been associated to cause liver injury in ruminants. The present study was designed to clarify, through malondialdehyde (MDA) determination in liver tissues, if administration of antioxidant supplementation reduces hepatic lipid peroxidation in bovines.

Materials and Methods: The study was conducted at the Veterinary School's farm named Tomé Pinto of the Federal University of Goiás, in São Francisco de Goiás, GO. A total of 40 Nelore bulls, aged between 24 and 36 months, were sorted into five treatments: G1 (non-supplemented control), G2 (10 mg of selenium and 1000 IU of vitamin E), G3 (600 mg zinc / animal - / day), G4 (10 mg selenium / animal / day) and G5 (1000 IU vitamin E / animal / day). The animals were fed twice a day and had water ad libitum. The diet followed the roughage and concentrate ratio 70:30. Animals were evaluated for 105 days and blood samples harvested on days 0, 56 and 105 for measurement of gamma glutamyl

transferase (GGT) and aspartate aminotransferase (AST) serum activity. Liver fragments were obtained through biopsies (0 and 56 days of confinement) and by harvesting liver tissue during the slaughter of animals at the end of 105 days of confinement.

Results: The animals showed no statistical difference for GGT and AST during the experiment ($p > 0.05$). No hepatocyte lipid peroxidation was detected by measuring MDA. Histological evaluation showed reduction in the quantitative changes in the liver parenchyma throughout the experiment. The following means were found for the parameters analyzed: TBARS (MDA mmol/Kg - G1: 9,09; G2: 6,88; G3: 7,96; G4: 7,17 and G5: 7,93); GGT (U/l) - G1: 20,24; G2: 20,61; G3: 20,45; G4: 21,41 and G5: 21,20) and AST (U/l - G1: 70,68; G2: 74,33; G3: 76,36; G4: 66,32 and G5: 76,75).

Conclusions: Among the antioxidants used as a supplement, the association of selenium with vitamin E showed the best results, minimized the occurrence of hepatic oxidative damage. Reduction was observed in the number of foamy macrophages in the hepatic parenchyma.

Nutrition & Metabolic Diseases

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Milk fatty acid profile with especial emphasis on Conjugated Linoleic Acid in bulk tank milk from Chilean farms with high and low levels of grazing

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Objectives: Conjugated linoleic acid (CLA) is a fatty acid (FA) of milk that is beneficial to human health. As a result, there would be benefits for human population if CLA content of milk is increased. There are several factors associated with levels of CLA in milk. Cows exposed to an intensive grazing of lush pastures, especially during winter-spring season, have been shown to produce a milk richer in CLA. Consequently, the objective of this study was to compare the profile of fatty acids in bulk tank milk between two dairy farms with high concentrate/ low pasture and low concentrate/high pasture based feeding

Materials and Methods: The study was conducted in two dairy farms from the south of Chile; one dairy was classified as high concentrate (6-8 kg/cow/day), low pasture based feeding (Dairy A) and the other dairy was characterized by low concentrate (1-2 kg/cow/day), high pasture based feeding (Dairy B). Dairy A consisted of 1,000 lactating cows with a herd average of 7,500 kg ME 305. Dairy B consisted of 700 lactating cows with a herd average of 4,000 kg ME 305. Both dairies had 70% of calvings in spring and 30% of calvings in fall. During October of 2012, 3 weekly bulk tank samples were obtained from both dairies. The milk samples were obtained during the morning milking in a plastic tube with preservative and submitted immediately after collection to the laboratory for fat content and FA profile analysis. The determination of milk fat (g/kg) was determined using infrared spectroscopy. Fatty acids were analyzed by Gas Chromatograph-Mass Spectrometry of butyl esters. Results were expressed as percentage relative to each FA detected in the chromatogram according to its retention time and identification based on pure standard. Differences in milk fat (g/kg) and FA (g/kg) content between the 2 groups were analyzed by ANOVA, developing a mixed model that also included average parity number, average days in milk and farm (A, B). Least squared means were estimated and tested for significant differences. Statistical analysis was performed using the

MIXED procedure of SAS (SAS Institute Inc., Cary, NC).

Results: Milk fat content was 3.45% and 3.78% for dairy A and B, respectively ($P < 0.05$). For fatty acid profile, average (\pm SE) conjugated linoleic acid were 1.25 ± 0.01 g and 2.06 ± 0.18 g for dairy A and B, respectively ($P < 0.01$). Omega-6/Omega-3 ratio was 6.38 and 3.1 for dairy A and B, respectively ($P < 0.01$). Total saturated fatty acids were 67.2% and 66.7% for dairy A and B, respectively ($P > 0.05$). Oleic acid levels were 21.3 ± 0.92 g and 16.05 ± 0.34 g for dairy A and B, respectively ($P < 0.05$).

Conclusions: It is concluded that conjugated linoleic acid and omega-3 fatty acids are higher in bulk tank milk samples from a dairy with low concentrate/high pasture based feeding system than a dairy with high concentrate/low pasture based feeding system in the south of Chile.

Nutrition & Metabolic Diseases

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Multi-mycotoxin analysis in silage – a survey from Central Europe 2015

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Objectives: Fusarium mycotoxins like deoxynivalenol (DON), zearalenone (ZEN) and fumonisins (FUM) are still among the most frequently occurring agriculturally relevant mycotoxins. The multi-mycotoxin approach allows not only the detection of these most prevalent mycotoxins in agricultural commodities. The occurrence of masked mycotoxins and fungal metabolites, which escape routine analysis posing a possible threat to the animal's health, can be targeted as well.

Materials and Methods: A total of 20 corn silage samples and 81 grass silage samples from Central Europe were analyzed with a multi-mycotoxin LC-MS/MS method at IFA-Tulln according to Vishwanath et al (2009). The analytical method was transferred to a more sensitive mass spectrometer (QTrap® 5500) and extended to cover more than 380 metabolites (Malachova et al, 2014; Streit et al, 2013). The accuracy of the method is monitored by regular participation in proficiency tests, which includes a separate testing scheme on "animal feed" (BIPEA, Gennevilliers, France).

Results: Deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEN) were detected in all 20 corn silage samples analyzed with average concentrations of 2,930 ppb DON, 128 ppb NIV and 958 ppb ZEN.

The masked forms of DON and ZEN, DON-3-glucoside and zearalenone-sulfate, also show a high prevalence in these samples. On average, a total of 37 metabolites were found in corn silage samples. Additionally, 80% of the corn silage samples were contaminated with fumonisins (FUM) at an average of 139 ppb. The main mycotoxins detected in grass silage were NIV, ZEN and fumonisin B₁ (FB₁) with average concentrations of 42 ppb NIV, 83 ppb ZEN and 29 ppb FB₁, respectively. Also the masked forms DON-3-glucoside and zearalenone-sulfate were detected in grass silage. On average, a total of 13 metabolites were found per sample analyzed. The most common metabolites detected in all grass silage samples were brevianamid F and cyclo (L-Pro-L-Tyr).

Conclusions: The present data highlights the high prevalence of

masked mycotoxins and metabolites that are not targeted by common analytical approaches. More data on the metabolic fate, mode of action, toxicity and possible synergistic interaction of co-contaminating mycotoxins is needed, to allow a risk assessment associated with the presence of these mycotoxins and other secondary metabolites found in silage samples.

Nutrition & Metabolic Diseases

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Monitoring Ketosis in dairy cattle: effect of two different therapeutic procedures on some metabolic parameters

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Objectives: The transition period in dairy cows is defined as 3 week pre-calving to 3 week post-calving and is the most critical phase of the lactation.

Ketosis is a common metabolic disorder that can appear during the transition period.

The aim of the present study was to evaluate the effects of two different therapeutic procedures used for preventive measures and treatment of ketosis on some metabolic parameters of lactating high-yielding dairy cows during transition period.

Materials and Methods: One-hundred-twelve Holstein Friesians dairy cows were recruited in two commercial dairy farms in North-East Italy. Cows were randomly divided into 3 groups.

Group 1: 52 dairy cows was treated with Acetyl-methionine, Inositol, Cyanocobalamin, L-alanine, L-arginine, L-threonine, L-glutamic acid (2 ml/10kg BW; IV administration; Bogras®, Ceva Salute Animale S.p.A.) at 12 and 9 days before the expected date of calving. At calving day animals were treated with Acetyl-methionine, Cyanocobalamin, thioctic acid, liver extract (20 ml per cow, 5 days; IM administration; Erbacolina®, Ceva Salute Animale S.p.A.).

Group 2: 42 dairy cows was treated with Monensin (335 mg; Oral Administration; Kexxtone®, Elanco Animal Health) 20 days before the expected date of calving.

Group 3: 18 dairy cows without therapeutic treatment (control group).

For each animal blood samples were collected from jugular vein and BCS was evaluated at 6 time points of sampling (-21±5, -7±5 days pre-partum; +7±5, +25±5, +50±5, +90±5 days post-partum). Metabolic parameters were analyzed: β-Hydroxybutyrate (BHBA), Non-Esterified Fatty Acids (NEFA), Glucose (GLU), Cholesterol (CHC), Triglycerides (TGR), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Gamma-Glutamyltransferase (GGT), Urea, Total Protein (TP), Albumin (Alb), Conjugated and Total Bilirubin.

Two-way ANOVA and post-hoc Bonferroni's multiple comparison test were performed to assess the statistically significant effects of the sampling time and treatments on the metabolic parameters.

Results: Two-way ANOVA showed different effects of treatments according to sampling time points.

Lower values of BHBA were recorded from 7 days pre-partum to 25 days

post-partum in both group 1 (0.44±0.18-0.73±0.36 mmol/L) and group 2 (0.43±0.16-0.64±0.19 mmol/L) than in group 3 (0.60±0.23-1.11±0.23 mmol/L).

Group 1 showed significant lower NEFA values from 7 days pre-partum to 90 days post-partum (0.48±0.22-0.60±0.26 mmol/L) than control group (0.85±0.27-0.75±0.18 mmol/L). Statistical lower NEFA value was recorded at 7 days pre-partum.

CHC was statistically higher in group 1 from 7 to 90 days post-partum (94.798±29.53-169.14±58.12 mg/dl) with respect to both group 2 (76.796±31.59-159.76±51.15 mg/dl) and control group (71.387±20.56-132.07±41.05 mg/dl).

At 7 days pre-partum and 7 days post-partum group 1 showed lower TGR levels (17.27±7.55- 2.69±6.48 mg/dl) than control group (24.61±7.96-18.38±4.61 mg/dl). Group 2 showed a decrease in TGR levels at 7 days post-partum (14.64±6.06 mg/dl).

Statistical lower Urea levels were recorded in group 1 from 7 to 50 days post-partum (24.17±12.31-26.44±13.69 mg/dl).

Conclusions: Our findings indicate that supplementation with the combination of acetylmethionine, cyanocobalamin and α-lipoic acid positively influenced liver metabolism in high-yielding dairy cows, indicating an overall improved energy status. This supplementation may be considered as a good therapeutic agent for the treatment and prevention of liver diseases.

Only limited effects on liver metabolism and BHBA can be obtained with monensin.

Nutrition & Metabolic Diseases

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Comparison of ruminal and blood gas variables of cattle induced to lactic ruminal acidosis throughout sucrose or oligofructose

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Objectives: Experimental induction of acute rumen lactic acidosis (ARLA) is traditionally carried out by infusing different types of sugar (mostly sucrose or oligofructose) into the rumen of cattle. Thus, we aimed to evaluate ruminal and blood gas variables after induction of ARLA by sucrose or oligofructose.

Materials and Methods: This study was approved local Animal Ethics Commission. Eight male, two years old Nelore steers were used. The animals were fitted with rumen cannula and weighted 398 kg in average. Cattle was divided in two groups of four animals according the experimental induction protocol: oligofructose group (OG) and sucrose group (SG). For induction of ARLA the OG received 17g of oligofructose per kg of body weight (BW). In this group cattle underwent an adaptation period receiving 5% of the total dose twice a day for three consecutive days before the induction. The SG received sucrose according to the formula $y = 1057 + 43,1 \times BW^{0,75}$, where y is the amount of sucrose in grams, being reduced 20% of the total dose. Ruminal and blood samples were taken at the following moments: before induction (T0), and after six

(T6h), 12 (T12h), 18 (T18h) and 24 (T24h) hours of the induction. Rumen fluid was used of pH measurement. Blood pH, carbon dioxide pressure (pCO₂), bicarbonate and basic acid deficit (ABE), were performed in the arterial blood through portable analyzer, using commercial cartridges (i-STAT, Abbott®, Abbott Park, U.S.A). Animals were treated after T24h for complete recovering. The distribution of all data was evaluated using the Kolgomorov-Smirnov test followed by repeated measures modelling with PROC-MIXED in the SAS.

Results: The animals from both groups showed ARLA (considered when ruminal pH was lower than 5.0) already in the T6h, reaching the minimum ruminal pH values 18 hours after induction. In the comparison between groups, the animals from OG had lower ($p < 0.05$) ruminal pH (OG = 3.56 x SG = 4.01) and blood bicarbonate (OG = 33.6 mmol/L x SG = 26.6 mmol/L) in T18h. It was also observed for OG lower blood pH values in T18h (OG = 7.14 x SG = 7.33) and T24h (OG = 7.14 x SG = 7.33) and lower ABE in T12h (OG = -22.5 x SG = -7.5), T18h (OG = -19.5 x SG = -6.0) and T24h (OG = 7.11 x SG = 7.39). There was no difference in pCO₂ between groups.

Conclusions: Cattle that received oligofructose for ALRA induction presented severe ruminal and metabolic acidosis. The oligofructose protocol induced to more acute and intense ruminal and metabolic acidosis than cattle that received sucrose.

Nutrition & Metabolic Diseases

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Effects of hyperketonemia within the first six weeks of lactation on milk production and fertility

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Objectives: The first 2 weeks after calving have been described as the main risk period for hyperketonemia (HYK). However, it was recently shown that dairy cows continued to be at risk for HYK until at least 42 DIM. The objectives of our study were to describe the epidemiology of HYK within the first six weeks of lactation and to evaluate the effects of HYK on milk production, reproductive performance and early lactation culling risk.

Materials and Methods: A total of 662 dairy cows from 6 commercial dairy farms in Germany were enrolled between 1 and 4 DIM. Cows were tested twice weekly using an electronic handheld meter for β-hydroxybutyric acid (BHBA) and glucose for an examination period of 42 days resulting in 12 test results per cow. Hyperketonemia (HYK) was defined as a BHBA concentration ≥ 1.2mmol/l. The onset of HYK was described as early onset (first HYK event within the first 2 weeks) and late onset (first HYK event in week 3 to 6 postpartum). Prevalence and incidence of HYK were calculated for the 12 examination days. The number of HYK events and time to a negative test event in HYK positive cows were described. The effects of onset of HYK within the first six weeks of lactation on milk production (1st test day milk yield and 100 DIM milk yield), reproductive performance (time to first service and first service conception risk), and on early lactation culling risk were analyzed separately using a GENLNMIXED procedure.

Results: Cumulative incidence of HYK was 47% and 69% for primiparous and multiparous cows, respectively. Multiparous cows had a higher incidence and prevalence of HYK within the first six weeks of lactation than primiparous cows. The peak incidence was found in the

first week of lactation and decreased steadily within the first six weeks of lactation. Prevalence stayed on the same level within the first six weeks of lactation. About 20% of all cows had one event of HYK within the first six weeks of lactation. Less than 5% of all cows had 6 or more HYK events. About 70% of HYK positive cows had a negative test result from one test to another 3 to 4 days later after the initial diagnosis of HYK. Cows with early onset of ketosis had a higher 1st test day milk yield (+2.3 kg/d, $P = 0.002$) and 100 DIM milk production (+287 kg; $P < 0.001$) compared to non-ketotic cows. There was no effect of late onset of ketosis on 1st test day milk yield and 100 DIM milk production. There was no effect of HYK on time to 1st service, irrespective of onset of ketosis and no effect of HYK on first service conception risk, irrespective of onset of ketosis. Cows with late onset of HYK had a decreased culling risk (HR = 0.365; $P = 0.045$) compared to non-ketotic cows. The culling risk of cows with early onset of ketosis was comparable to non-ketotic cows (HR = 1.447; $P = 0.212$).

Conclusions: The risk period for HYK lasted at least until lactation week 6. However, a high number of HYK positive cows had a negative test result after the initial diagnosis of HYK. Cows with early onset of HYK had higher milk production compared to non-ketotic cows. HYK was not associated with negative effects on reproduction and early lactation culling risk. HYK in early lactation seems to be part of a physiological adaptational response to negative nutrient balance in transition dairy cows.

Nutrition & Metabolic Diseases

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The prevalence of subclinical hypocalcaemia on a high producing commercial Dutch dairy farm and the effect of oral calcium supplementation

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Objectives: In this field-case study the prevalence of clinical and subclinical hypocalcaemia was determined at a large commercial Dutch dairy farm with a high average milk yield. Furthermore the effect of supplementation of cows with oral calcium boluses after calving on the total blood calcium concentration was evaluated. This study was initiated and executed by a veterinary practice in the Netherlands.

Materials and Methods: The herd consisted of 366 Holstein Friesian lactating cows. The average milk yield was 11270 kilograms milk per 305 days. The cows are kept indoors year-round and fed a mixed ration. In the milking parlour the cows are fed concentrates. The dry cow period last on average 35 days and the cows are housed in two groups. Both groups are given silage, maize, hay, brewers grain and dry cow minerals. The close-up group, two weeks before calving, are housed in a large pen with straw bedding. No anionic salts or special concentrates are used in the dry cow ration. Within 4 hours after calving whole blood samples were collected via venepuncture of the coccygeal vein into a serum vacutainer. Total calcium (mmol/L) was measured using the Medonic Spotchem (A. Menarini diagnostics). After the collection of this first blood sample the cow was supplemented with an oral calcium bolus (Bovicalc Boehringer Ingelheim), between 12-24 hours after the first bolus the cow received a second Bovicalc bolus. Between 24-48 hours after the second Bovicalc bolus was given a consecutive blood sample was taken, following the same procedure as the sampling directly after calving. In total 58 cows were sampled, 9 cows (15.5%) with first parity (heifers) and 49 cows (84.5%) with a higher parity. Because of practical reasons only 18 cows

were sampled twice, so before and after treatment with a Bovikalc bolus. From this group 5 cows were excluded from analyses because they also received a calcium infusion.

Results: The cut-off values for subclinical hypocalcaemia was set on 1.381 (49 animals) had an average total calcium concentration of 1.92 mmol/L with a standard deviation of 0.34. The percentage of cows with a parity >1 that had normo-calcemia subclinical hypocalcaemia or clinical hypocalcaemia was respectively; 36.7%, 57.2% and 6.1%. The group of cows (13 animals, all parity >2) that were sampled twice and were treated with a Bovikalc bolus had an average total calcium concentration at first sampling of 2.03 mmol/L with a standard deviation of 0.48. After treatment the cows had a total calcium concentration of 2.21 mmol/L with a standard deviation of 0.50. There was difference, close to statistically, between the total calcium concentrations before and after treatment (p -value = 0.06).

Conclusions: This field-case study shows that on a large well managed dairy farm in the Netherlands the prevalence of hypocalcaemia is high (63.3%). A treatment protocol with Bovikalc boluses in a small group of cows around calving and 12-24 hours later increased the total calcium concentration from subclinical to normal levels. A whole herd treatment will be beneficial on this farm and further studies will be done on the effect of this strategy on disease prevalence after calving in this particular herd.

Nutrition & Metabolic Diseases

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Primary bovine hepatocytes from abattoir derived liver as in-vitro model for determinants of growth hormone receptor down-regulation

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Objectives: The somatotrophic axis uncouples during the transition period; meaning the growth hormone receptor expression (GHR) in the liver decreases. Next to insulin, other factors are known to influence the hepatic GHR-expression and -signal transduction (e.g. thyroid hormones, sexual steroid hormones, fibroblast like growth factor 21 etc.). The aim was to establish a long-term primary hepatocyte cell culture from bovine abattoir derived liver expressing the growth hormone receptor (GHR) in a sandwich culture system in order to study the effect of single endocrine, immune and metabolic factors affecting the GHR in future.

Materials and Methods: The caudate lobe of the liver was taken 20 min after slaughtering, rinsed by ice cold Eurocollins buffer (Giantin et al. 2012), and after 2h transport to the laboratory on ice, the liver was re-warmed for five minutes at room temperature. Thereafter, cannulas were inserted in four vessels at the cut surface and fixed by tissue glue. A three-step-collagenase perfusion was performed until the extracellular matrix was abrogated. After washing, viable hepatocytes were separated using Easycoll™ and viability was proven by Trypan blue staining. The cells were seeded on collagen coated plates with Williams E Medium containing 10% FCS. After 2 h adhesion, medium was replaced and the cells were either cultured (without FCS, 37°C, 5% CO₂) for 4 d in a monolayer (ML) or with a collagen overlayer as a sandwich culture (SW) for 10 d. After establishing this protocol, the procedure was repeated (8 times), and cell samples for mRNA-expression of apoptosis markers (Bax, Bcl2-xL, Fas Ligand) and for GHR1A and IGF-1 mRNA expression

were taken, as well as medium samples for urea measurement. The viability during the cell culture was proven by using the Live and Dead Cell Staining Kit (Abcam, Cambridge, UK).

Results: The mean viability was 86 ± 7 % after purification. In ML and SW, the cells changed morphologically from roundish-spherical to polygonal hepatocyte like cell shape and build cell-cell contacts. The mRNA-expression of apoptosis markers stayed low during the separation procedure and during culture ($P < 0.05$). The urea production was higher in ML than in SW culture during the first three days, and in both urea concentration in the medium decreases during the first three days. In SW culture hepatocytes stayed vital with continuous urea production until day 10. The GHR-expression (mean \pm SD, 48.4 ± 7.3) decreased after purification (30.9 ± 9.6 ; $P = 0.0155$), whereas IGF-1 expression stayed constant (2.87 ± 0.94 vs. 2.81 ± 1.10 ; $P = 0.9288$).

Conclusions: In conclusion, viable hepatocytes can be obtained from abattoir derived bovine liver and cultured for 10 days in sandwich-culture. This promising in-vitro model of bovine hepatocytes can be used to characterize single factors affecting the GHR-expression in dairy cows. Next to the GHR-expression in future experiments the functionality of the receptor should be proven by addition of bovine GH.

Nutrition & Metabolic Diseases

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Development of wireless sensors for the measurement of rumen milieu parameters in cows

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Objectives: For further experimental purposes a wireless device for continuous measurement physico-chemical parameters the ruminal milieu was developed at the Animal Science Institute in Prague along with the Department of Cybernetics of West Bohemian University, Czech Republic. The aim of this study was to test the reliability of the device during a one month continuous measurement in situ on dry fistulated cows.

Materials and Methods: Autonomous sensors were developed for the in situ continuous measurement of ruminal milieu parameters (temperature, pH, redox potential-Eh). The probes are placed in the rumen of cows fitted with ruminal canula. The probe sinks in the liquid phase of ruminal content due to the weight it is fitted with. The parameters are measured at adjustable pre-defined intervals (2 minutes in this study). The values measured are transferred by radio signal to the receiver placed in the barn and are shown in real time on the display of computer with a special user interface. In addition the data are stored in the flash memory inside the ruminal device. With the measurement interval of 2 min, data of 6 month measurement can be stored. Temperature is measured by a chio integrated sensor with 0.25°C accuracy. pH and redox potential are measured with glass electrodes attached to sensitive amplifiers and analogue filters integrated inside the ruminal device. pH and Eh measurement accuracy is 0.002 pH and 0.27 mV, respectively. A special software has been developed along with the device enabling decimation and smoothing of the values and creating curves.

The device prototype was placed in the ventral ruminal sac of a cannulated dry cows that received the diet consisting of alfalfa haylage, maize

silage, pea silage, barley straw and meadow hay. The measurements were performed continuously for 28 days. Feed was given twice a day, at 6 a.m. and at 2 p.m.

Results: During the measurement period no significant displacements of the device in the rumen were noted.

The mean pH values were ranging within 5,8 – 6,9. There is a marked drop in pH after the morning feeding and increase at night when the cow did not ingest much feed. These results correspond with the data reported by many other researchers.

The pH drift on the pH sensor after 28 days of continuous measurement was 0.2 pH.

The mean Eh values were ranging from -210 mV to – 150 mV and the course of the diurnal curve corresponded with the data reported in the literature.

Redox potential was increased postprandially, after the morning feeding, probably due to penetration of air oxygen with ingested feed into the ruminal milieu. The subsequent drop in Eh is due to a fast consumption of oxygen by ruminal microflora.

Because this study tested the function of the device, laboratory analyses of rumen fluid were not performed, these were included in further studies.

Conclusions: The technology of measuring ruminal parameters by an indwelling device with wireless transmission and storage of data measured enables continuous monitoring of the ruminal milieu. Such data can help evaluate the effect of diet on ruminal fermentation, stability of ruminal microflora and the impact on the health status and performance of the cow.

In the current form the method is suitable for research purposes. The next aim is to miniaturize the devices to be suitable for the use under farm conditions.

Nutrition & Metabolic Diseases

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The Biogenic Amine Gaba (Gamma Aminobutyric Acid) In Grass Silages. Part 1 Examination Of The Gaba Formation And Concentration

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Objectives: In the Netherlands, Germany and Denmark, more than 90% of the locally produced forages are stored as silage (Oude Elferink, 1999). During ensiling, one of the most significant processes in grass silages is the degradation of true protein (tp) to soluble non-protein nitrogen components (NPN) by enzymatic breakdown. In fermented forages, the NPN fraction includes peptides, free amino acids (AA), amides, amines and ammonia with free AA making up the largest part (van Soest, 1994). The free AA can undergo an anaerobic decarboxylation through microbial enzymes to biogenic amines, e.g. glutamic acid to γ-aminobutyric acid (GABA).

Materials and Methods: In Lower Saxony, near the German North Sea coast, pasture for grazing cattle or winter feed is the dominant use of the grassland, and there are mainly dairy farms in this area. Since

approx. 1996 an unspecific disease with unknown origin is observed in dairy herds of our region with the following clinical signs: 1.) beginning with lameness, 2.) followed by weight loss, 3.) cachexia and sometimes 4.) sudden death. The protein quality of the grass silage in dairy cow's rations is suspected to be the reason for this unspecific disease. To get more detailed informations, we investigated 648 grass silage samples from bunker silos in dairy farms with and without problems since 1996. Comparing the silages of farms having either a high or a low incidence of ill cows in their herds, there was only one common finding: reduced concentrations of tp in crude protein (cp) and an increased amount of NPN in the grass silages (Heimbeck et al., 2002; Coenen et al, 2004; Eicken 2005a). Because free AA make up the largest part of the NPN fraction in fermented forages, we looked for more details in the chemical composition of these silages with strong proteolysis, and analyzed a considerable increase of free AA. Particularly the GABA concentrations of 248 samples showed the following **Results:** 1 - 3 g/kg DM in grass, hay, maize silage and 2.5-15 g/kg DM in grass silages (Evonik Nutrition & Care, Hanau).

Results: Normally a grass silage with little protein breakdown contains 70% tp and 30% NPN-compounds in the cp. Between 1996 and 2000 the percentage of grass silages with tp concentrations of <40-50% in cp showed a remarkable increase. The main part of anaerobic decarboxylation of glutamic acid during fermentation is done by glutamate decarboxylase (GAD) (Syragusa et al., 2007) and leads to GABA. This chemical reaction is irreversible (Cagno et al., 2010) and can only be done by microbial enzymes (Lu et al., 2009). Especially in first cut silages higher GABA concentrations were determined accompanied by a strong tp degradation (to less than 40 – 50% in cp). (GABA detection: Commission Regulation[EC] No 152/2009 of January 2009, laying down the methods of sampling and analysis for the official control of feed). High GABA concentrations in the grass silage were only found in the herds with the unspecific clinical signs. Since several years, we studied and documented the situations in similar dairy herds of our region. The GABA concentrations of the grass silages varied between 3 to 15 g/kg DM. On many farms the rations for dairy cows contain 10 kg DM grass silage/cow/day. So the resulting GABA intake ranges between 30 to 150 g/cow/day. This is well documented for the past 8 years. Based on our experiences we assume that 40-45 g GABA/cow/day will be tolerated by the cows.

Conclusions: Since approx. 1996, an unspecific disease of unknown origin is observed in dairy herds in Northern Germany with the following clinical signs: 1.) beginning with lameness 2.) followed by weight loss 3.) cachexia and sometimes 4.) sudden death. High GABA intake with grass silage is suspected to be the reason for this unspecific disease in the dairy herds. If the GABA intake of a dairy cow (especially in peak lactation) exceeds 45 g/day for several weeks, the dairy farmer can expect a severe health risk in his herd.

Nutrition & Metabolic Diseases

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Feeding Management Practices During The Transition Period - Its Effect On Disease Incidence In Dairy Farms In The Riverina Region Of Nsw, Australia

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Objectives: 1) To identify the transition management practices currently adopted by dairy farmers in the Riverina dairying region, 2) to determine serum concentrations of calcium (Ca), magnesium (Mg), Non-esterified fatty acids (NEFA) and Beta Hydroxy Butyrate (BHOB) within the postparturient period, and 3) to assess the relationships between the transition management practices, serum metabolite concentrations, and incidence of metabolic disorders such as ketosis (SCK,CK), milk fever (MF) and retained placenta (RP) and within the first 40 days in milk (DIM).

Materials and Methods: Five participatory dairy farms out of 10 farms in the Riverina dairying area were chosen for this study. Both feeding and husbandry practices during the dry period, immediately following parturition and during early lactation currently adopted in each of the farms were recorded. Blood samples were collected from a representative sample of cows from each farm at +3 (D3) and +35 (D35) post calving. The serum separated and analysed for serum Ca, Mg, BHOB, and NEFA concentrations. Occurrence of SCK, CK, MF and RFM within the first 40 DIM was also recorded.

Results: Transition management practices during the dry period varied between the farms. Three farms had a dry period length of 5 to 6 weeks while 2 farms had the traditional 8-week dry period. Farms 1 and 2 used solely ad lib cereal hay in their transition cow rations, fed for approximately 3 weeks prior to expected calving date. Farms 3, 4 and 5 used a professionally formulated pelleted transition supplement containing anionic salts and monensin, fed with ad lib cereal hay. All farms were pasture based post calving but in addition to pasture all farms on an average fed 4 to 8.5 kg/cow/day of concentrates only Farm 5 fed 3.5 kgDM/cow/day of silage in addition. Regardless of the farm there was a significant ($P < 0.05$) difference in the serum levels of Ca, Mg, BHOB, NEFA between D3 and D35, levels at D3 were lower compared to D35. While serum electrolyte levels improved, NEFA and BHOB were higher at D35 indicating a greater degree of NEB with an increase in prevalence of SCK by D35. The serum Mg level on D3 was found to be significantly higher on farms that were fed anionic salts in the dry period. Feeding of hay ad libitum during the dry period resulted in higher mean serum Ca postpartum ($P < 0.05$). Early lactation feeding practices were found to significantly affect ($P < 0.05$) serum Mg on D3. Farms 2 and 3 had significantly lower serum Mg than Farm 4 ($P < 0.05$), Farm 4 fed a high level of concentrate during early lactation compared to other farms. Over sixty percent of cows suffering from milk fever or SCK succumbed to another disease (ketosis, RFM, metritis) event within the study period.

Conclusions: The results of the study has shown that there is a high level NEB experienced by cows in the peri parturient period which could result in a high incidence subclinical and clinical metabolic disorders within the farms in the Riverina region, which has implications on both the welfare of animals and farm profitability. The results have also shown that farms adopting good transition management practices such as having a shorter dry period and supplementation closer to calving decrease the risk of developing metabolic disorders during the peak lactation period.

Nutrition & Metabolic Diseases

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Estimation Of Glucose Utilization By Peripheral Tissues In Healthy And Ketotic Cows During An Intravenous Glucose Tolerance Test

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Objectives: To estimate functional state of endocrine pancreas in healthy and ketotic cows during intravenous glucose tolerance test.

To estimate blood glucose utilization by peripheral tissues in healthy and ketotic dairy cows on the basis of changes in blood concentrations of glucose, insulin and inorganic phosphorus during intravenous glucose tolerance test.

Materials and Methods: Eight healthy and seven ketotic cows in the earliest stage of lactation were chosen from a Holstein dairy herd. The test was carried out in the morning at 9 a.m. about 3 h after feeding. A solution of glucose (500 ml of 50 %) was injected intravenously over a 5-minute period into the jugular vein of each animal. Blood samples were taken from the opposite jugular vein before (0) and 10, 30, 60, and 90 minutes after injection. The concentration of insulin in the blood serum was determined by ELISA methods (Cusabio) using a Rayto reader. Glucose and inorganic phosphorus were measured by GOT method using a Radox kit and Rayto spectrophotometers. The effect of time after intravenous glucose tolerance test on glucose, insulin and inorganic phosphorus levels was analyzed by ANOVA-procedure with a post hoc LSD test. The intensity of metabolic change during intravenous glucose tolerance test was analyzed by determining the difference in the change of glucose, insulin and inorganic phosphorus concentrations (conc. at 30 min – conc. at 0 min, t_{30-t_0}) in healthy and ketotic cows using a t-test. The relationship between the change in insulin and glucose concentrations and the change in inorganic phosphorus concentration was assessed by the simple linear regression model. The statistics software Statgraphic Centurion (Statpoint Technologies Inc. Warrenton, Va, Virginia, USA) was used.

Results: After intravenous infusion of glucose solution, blood glucose and insulin values in both groups of cows increased significantly within 10 and 30 minutes of the experiment ($p < 0.05$). In ketotic cows, mean blood insulin and glucose levels were significantly lower than in healthy cows during 10, 30 and 60 minutes of the experiment ($p < 0.05$). After intravenous infusion of glucose, inorganic phosphorus levels decreased in blood ($p < 0.05$) in both groups of cows. Statistically significant lower ($p < 0.05$) values in blood inorganic phosphorus were determined in ketotic cows than in healthy cows during the test period. The increase in insulin and glucose concentrations and the decrease in inorganic phosphorus concentration (30 minutes - 0 minute) were statistically higher ($p < 0.05$) in healthy cows compared with ketotic cows. A strong correlation was established between blood glucose, insulin and inorganic phosphorus levels in both groups of cows, with significantly ($p < 0.01$) higher changes in the blood parameters found in healthy cows than in ketotic cows.

Conclusions: Low insulin secretory responses in ketotic cows are likely due to the low pancreatic capacity for insulin secretion that develops during the days or weeks of malnutrition-induced hypoglycemia which is generally accompanied by ketosis. The statistical analysis revealed a strong correlation between blood glucose, insulin and inorganic phosphorus levels in both groups of cows, with significantly higher changes in the tested blood parameters in healthy cows than in ketotic cows. The results suggest a higher degree of blood glucose utilization by peripheral tissues in healthy cows during the intravenous glucose tolerance test.

Nutrition & Metabolic Diseases

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Effect Of The Supply Of Viusid Vet On The Productive Behavior Of Mestizos Holstein X Cebú Calves Of Different Ages

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Objectives: You can not aspire to an efficient and productive livestock to not achieving adequate development of calves in the early stages of life, which is closely related to his subsequent behavior. The objective of this essay was evaluate the influence of the supply of VIUSID vet used as a food additive on the bio productive behavior of Holstein calves and mestizos Holstein x Cebu calves of different ages in an artificial breeding system in Cuba

Materials and Methods: The study was carried out in the artificial breeding center of the "Dos Rios" Genetic UEB, of the province of Sancti Spiritus, Cuba. For this, four experiments were carried out on a total of 234 animals.

VIUSID vet contains: Malic acid, Glucosamine, Arginine, Glycine, Ascorbic Acid, Folic Acid, Monoammonium Glycyrhizinate (extracted of the root of Glycyrhiza glabra), Pyridoxine Hydrochloride, Cyanocobalamin, Calcium Pantothenate and Zinc Sulfate. The product undergoes a biocatalytic process of molecular activation to improve their biological activity and the biochemical reactivity of all their molecules.

In experiments 1 and 2, 59 and 60 calves were used respectively, which were younger than 2 months, and in experiments 3 and 4, 60 and 55 calves were used respectively, with ages between 4 and 5 months. All experiments were performed in different ships. In all the trials, the animals were divided in 3 homogeneous groups taking into account age, body condition and breed. The treatment applied was as follows: group I was used as control group, groups II and III received 2 g and 4 g of VIUSID vet, along with milk or concentrated, depending on age for 35 days. The variables studied were: initial weight (baseline), final weight (35 days trial), weight gain, average daily gain, hematocrit, hemoglobin, incidence of diarrhea, mortality and its causes, incidence of other diseases

Results: It was observed that the average increase of weight per calf, in the first experiment was significantly higher ($p < 0.05$) than 5.9 kg and 8.3 kg in groups II and III respectively, in regards to the control group; in experiment 2, the average weight increase was higher in 7.8 kg and 15.6 kg in the groups II and III respectively in regards to the control group; in the third experiment, the calves gained 7.5 kg and 10.5 kg more, in the groups II and III respectively in regards to the control group, and in the experiment 4, the average weight increase per animal was higher in 6.6 kg and 5.3 kg in groups II and III respectively in regards to the control group. No significant differences were found in any of the experiments in the results of the hematological studies or in the incidence of disease and deaths between the studied groups.

The better behavior of the animals can be given by the effect of product composition, which may be supplementing important elements necessary for the growth and development functions of these animals, especially if one considers that the molecular activation is performed at the same effects that it incorporates no components by themselves

Although no significant differences between the animals under study, in the treated groups there was a slight increase in hematocrit and hemoglobin at the end of the experiment, which allowed the group III

minimum values reach normal hematocrit, not the case in the Control group, this value Decreased, sharpening anemia presenting more animals

Conclusions: In experiments where animals younger than 2 months of age were used, there were significant differences between the treated groups compared to the control, but not between the two treated groups

In experiments where animals are aged between 4 and 5 months, the group receiving 4 grams of Viusid, It behaved significantly better than receiving 2 grams and both groups performed better than control

No significant differences were found in terms of hematological parameters between groups

Nutrition & Metabolic Diseases

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Ruminal phenolic content and impact of soybean meal by feeding grass silages containing different levels of true protein in vitro

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Objectives: Grass silages containing < 50 % true protein (TP) in total crude protein (CP) are suspected to cause a clinical picture in dairy herds called 'Faktorenerkrankung Milchviehherde'. In practice soybean meal improved the symptomology. Previous studies in vitro have shown an increase of phenolic compounds in bovine ruminal fluid when suspected grass silages (TP/CP < 50 %) were fed. An in vitro study was conducted to assess the effect of soybean protein on the ruminal content of total phenolics and o-diphenols during fermentation of grass silages with TP/CP < 50 %.

Materials and Methods: In the experiment 2 x 4 runs with duration of 28 days a time were conducted using the RUMen Simulation TECHnique (RUSITEC). Two different grass silage pairs were tested in 4 RUSITEC runs each (R1-4 and R5-8). During the adaptation and control period (9 days) grass silages with TP/CP > 50 % (CS) and corn starch were fermented in all three fermenter groups (control, I, II). In the following experimental period (10 days) fermenter groups I and II were fed grass silages containing TP/CP < 50 % (TS) and group II with addition of soy protein. During the end period (9 days) the initial diet was added to all fermenters again. The two grass silages compared in each trial were harvested from the same fields. In daily taken samples of ruminal fluid the total phenolic content was measured using the semiquantitative colorimetric Folin-Ciocalteu method. The content of o-diphenols was quantified with a semiquantitative colorimetric method after performing an alcoholic extraction.

Results: The contents of total phenolics and o-diphenols stabilized in all eight trials during the adaptation and control period. A change of grass silages fed by day 9 resulted in a significant shift of detected phenolic quantities. The effects of TS addition were most visible on days 15 to 19. At that time the total phenolic content was lowered by addition of TS in R1-4 (group I: -20,7 %; group II: -19,7 %; $p < 0,001$) and increased in R5-8 (group I: +12,8 %; group II: +12,1 %; $p < 0,001$), respectively. Simultaneously o-diphenol levels declined in R1-4 (group I: -5,11 %; group II: -6,24 %; $p < 0,001$) and at a lower extend in R5-8 (group I: -1,89 %; $p < 0,001$; group II: -2,31 %; $p < 0,01$). The soy protein

supplementation had no noticeable effect. After switching back to the control ration on day 19, the concentrations of phenolic compounds aligned with the control again within four days. The control values were consistent during the whole trial.

Conclusions: It could be shown that grass silages with TP/CP < 50 % had significant effects on the phenolic composition of the ruminal fluid. The mechanism of the beneficial effect of soybean meal seen in practice could not be elucidated in this study. Although there was no explicit monodirectional change in the total phenolic content identifiable, it appears likely that the phenolic composition of a diet can influence the ruminal digestion.

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Nutrition & Metabolic Diseases

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The effect of autolysed yeast supplementation on in vitro rumen fermentation in high versus low fiber rations

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Objectives: Autolysed yeast is the result of a standardized degradation of the yeast cell by its own enzymes, providing immediately available functional components, which have a prebiotic effect on rumen microbiota.

The objective of this study was to test the effects of an autolysed yeast, Levabon® Rumen E, in high-fiber ration (HFR) and low-fiber ration (LFR) in two independent in vitro rumen simulation trials.

Materials and Methods: The in vitro trials were carried out using the rumen simulation system (RSS), at the BIOMIN Research Center (Tulln, Austria). The RSS allows continuous fermentation in 12 individual reactors for 15 days at 39 °C, under constant stirring and inflow of synthetic saliva. In two consecutive experiments the reactors (total volume of 1.5 l) were inoculated with rumen fluid and solid digesta from slaughtered animals and fed daily with 20 g of total mixed ration. Forage to concentrate ratio in LFR was 30:70 and in HFR 55:45 on dry matter basis.

In each of the two rumen fermentation trials six reactors received LFR and the other six HFR. Three reactors per diet received autolyzed yeast (*Saccharomyces cerevisiae*, Levabon® Rumen E) on top of their ration at a level equivalent to 30 g/animal/day. Fresh feed was provided once a day and remained in the reactors for 48 hours. After a one-week adaptation period rumen fluid was sampled for analysis of volatile fatty acids (VFAs) and bacterial abundance via cannula from each reactor. VFAs were analyzed by high performance liquid chromatography (HPLC); bacterial counts were determined using flow cytometry.

For statistical evaluation general linear models were calculated in SPSS version 19 with trial number as co-variable. The level of significance was $p < 0.05$.

Results: Concentrations of acetate and propionate were higher in the LFR compared to HFR for both, control and Levabon® Rumen E groups. Although Levabon® Rumen E supplementation in the LFR increased acetate and propionate production in both trials; this was only significant for acetate at 360 hours of fermentation. The acetate-propionate ratio

was significantly higher in reactors receiving HFR compared to LFR diet in both groups. Higher bacterial counts were observed in the Levabon® Rumen E supplemented group in both LFR and HFR ration. Levabon® Rumen E supplementation increased on average acetate production by 12%, propionate by 13% and bacterial counts by 35% on LFR and also increased acetate by 1%, propionate by 6% and bacterial counts by 13% on HFR.

Conclusions: A significant increase of the acetate-propionate ratio in HFR compared to LFR was observed in vitro. This corresponds well to results of a meta-analysis of in vivo data, stating that cellulose supplementation increases the acetate-propionate ratio, while starch and soluble carbohydrates typically lead to a lower ratio (Murphy et al, 1982).

Levabon® Rumen E supplementation stimulated VFA production and bacterial counts in both rations; however, it had a particularly strong effect in LFR. This positive influence was observed reproducibly in two independent in vitro trials, using rumen fluid from different animals as inoculum.

Comments: Literature

Murphy, M. R., R. L. Baldwin, and L. J. Koong. 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. *J. Animal Sci.* 55:411-421.

Nutrition & Metabolic Diseases

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Dietary Supplementation With Organic Chromium Mitigates Weaning Stress In Beef Calves

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Objectives: Weaning is one of the most stressful episodes in the lifetime of a calf and the lactating cow. There are several changes in the management of weaning to decrease the outcome stress. Most of them depends on the handlers and the farmer pawns no so easy to manage. Thus, other factor independent of these personals are required. One possibility is the use of organic chromium that in stressful situations brings beneficial results to the animals. The objective of this experiment is to evaluate the use of organic chromium to mitigate the stress of weaning.

Materials and Methods: An Official Brazilian Animal Ethics Commission approved this protocol, previously.

One hundred and fifty crossbred six-month beef male (74) and female (76) Nellore and Nellore crossbred calves were used. These calves were distributed into two equal groups and both received a corn, soybean meal, mineral supplement on a 0.1% BW daily basis, in a creep feeding, throughout the 60 d before and 60 d after the weaning. It was included in the diet of one group 0.9 mg Cr/100 kg BW of organic chromium. Body weight was measured and samples were collected at different times (day 0, weaning, 48 h after weaning (48W) and at the end of the experiment) to measure serum cortisol, glucose, total protein and albumin, chromium, and urinary chromium and creatinine. At 48 W the temperament of the calves was assessed by the scale composite score to classify them as

calm or stressed calves. The distribution of all data was evaluated using the Shapiro-Wilk test followed by repeated measures modelling with PROC-MIXED in the SAS. The Qui-square test was also performed.

Results: The chromium supplementation increased significantly the weight gain before weaning, with an (average of 5.6 kg), and throughout the experiment (6.5 kg); decreased the number of stressed calves (24/75 and 6/75), and the serum levels of cortisol (76.5 x 63.7 nM cortisol) (P <0.01). The weaning stress caused an increased urinary excretion of chromium(498.5 x 336.7 nM Cr), independent of Cr supplementation, and the urinary index of excretion of chromium (24.5 x 9.5 nM Cr) being highest in the stressed calves with high levels of cortisol (P = 0.04).

The results showed that weaning stress could deplete the reserves of body chromium by increasing its urinary excretion, but the dietary supplementation of Cr by creep feeding can overcome this depletion resulting in a lower production of cortisol and a less stressful weaning, since Cr modulates the production of cortisol.

Conclusions: The detrimental effects of weaning triggered in the most stressed calves higher levels of serum cortisol, greater excretion of urinary chromium and decreased their weight gain throughout this time, but this condition can be mitigate and corrected in most calves by dietary supplementation of organic Cr.

Nutrition & Metabolic Diseases

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Influence of GLP-1 and GLP-2 on the epithelial barrier of the ovine rumen

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Objectives: The epithelial barrier of the ruminal epithelium is provided by a tight junction protein (TJ) strand network sealing the space between contiguous cells. It constitutes the intercellular fence against solutes, microorganisms and toxins. In jejunal epithelia of monogastric organisms, it has been shown that the epithelial barrier can be tightened by glucagon-like peptide 2 (GLP-2) (1). On the other hand, glucagon-like peptide 1 (GLP-1) decreases endothelial permeability in injured mesenteric endothelium (2). Against this background, we addressed the hypothesis that GLP-1 and GLP-2 may also affect the epithelial barrier of the ovine rumen.

Materials and Methods: Six adult sheep were fed on standardized 80% hay and 20% concentrate diet for at least 2 weeks prior to the experiment. Sheep were stunned and exsanguinated, and ruminal tissue was removed from the ventral ruminal sac. The epithelial layer was stripped off the *tunica muscularis* and *tunica serosa*. Epithelia were cut in ~3x3 cm pieces and mounted in Ussing chambers with an exposed area of 3.14 cm². The incubation buffer on the mucosal side was titrated to pH 6.1 and contained short chain fatty acids (SCFA; 40 mM). The incubation buffer at the serosal side had a pH 7.4 and was SCFA-free. Epithelial conductance was continuously monitored under short-circuit conditions. One control group was incubated for 7 hours in buffer solution without hormones. Simultaneously, four treatment groups were incubated on the serosal side with incubation buffer containing either 25 nM or 250 nM of either GLP-1 or GLP-2. After 7 hours, epithelial samples were collected and stored in RNA later. The changes in mRNA expression of the TJ proteins claudin-1, -4, -7 and occludin were measured by reverse transcription quantitative real-time

PCR (RT-qPCR). Statistical analysis was performed with the software SPSS 22 using one-way ANOVA and the post-hoc Dunnett test.

Results: The electrical conductance was not different between control epithelia incubated without hormones and epithelia incubated with GLP-1 or GLP-2 at the two tested concentrations. However, the mRNA patterns of tight junction proteins were different between epithelia incubated with and without hormones. The mRNA expression of claudin-7 decreased in all epithelia incubated with GLP-1 at 25 nM (by -75 ± 7%) or 250 nM (by -82 ± 8%), as well as GLP-2 at 25 nM (by -56 ± 10%) or 250 nM (by -57 ± 9%), when compared to control expression (all P < 0.05). The mRNA expression of occludin increased only in epithelia incubated with 250 nM of GLP-2 (by +239 ± 75%; P < 0.05) but was not different to controls in the presence of 25 nM or 250 nM GLP-1 or 25 nM GLP-2. The mRNA expression of claudin-1 and claudin-4 was not different between epithelia incubated with or without hormones.

Conclusions: We conclude that both GLP-1 and GLP-2 decrease the mRNA expression of the tight junction protein claudin-7. Very high concentrations of GLP-2 additionally increase the mRNA expression of occludin. These changes may be relevant for adaptation processes of the ruminal epithelium in the longer term but may not be associated with immediate changes in epithelial barrier function.

Comments: This study was supported by an Elsa Neumann stipend of the state of Berlin to G. Greco.

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Nutrition & Metabolic Diseases

P01-001-071

Changes in Several Serum Microminerals (Cu, Zn, Mn And Se) Concentrations associated to Management System in Dairy Cows in Galicia

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Objectives: This study aimed at: i) investigating the effect of the management system (organic vs. conventional) on the serum concentrations of Cu, Zn, Mn and Se; and ii) studying the temporal changes of these minerals around the time of calving

Materials and Methods: This study was conducted in 3 herds, two organic (considered as an experimental group, where animals don't receive any kind of mineral supplementation) and one intensive farm (where animals receive a mineral supplementation in the diet), located nearby in Galicia (NW Spain). Blood samples were obtained at different time points by coccygeal venepuncture from 41 organically managed HF cattle (21 from organic herd A, 20 herd B) and 37 intensively managed cattle. Sampling took place at the dry-off (60-30 days before expected calving), close-up (29-3 days before expected calving), fresh (3 to 30 DIM) and peak lactation (31 to 60 DIM) stages, as well as during mid-lactation (180-210 DIM). Serum concentration of Cu, Zn, Fe and Se were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Data were analysed statistically through the GLM procedure

of SPSS v20.0, including the fixed effects of management regimen, productive stage and their interaction. Post-hoc analysis was conducted with the Bonferroni test.

Results: The majority of the serum parameters, except Se, were with physiological ranges. Considering the management system, the lower concentration of Se was lower in the organic herds, whereas the concentration of Mn and Zn was higher in the organically managed cattle. For serum Mn and serum Zn, organic herds showed higher and statistical different levels than intensive herd. Considering the productive stage, statistical differences were only observed in one of the organic herd (herd A) between the mid- and early lactation stages. Serum Zn was lower during the fresh period both in the intensive and the organic herd A, increasing its concentration during peak of lactation. Finally, with regards to the serum concentration of Cu, it was lower in the organic farms; and considering the productive stages, the highest concentrations were found during the lactation peak and the lowest during close-up in both the intensive and the organic herd A. No differences throughout the study period were found in the organic herd B.

Conclusions: The changes over time around calving of the serum concentration of Cu, Zn, Mn and Se were not different between organically and intensively managed dairy cattle. Indeed, in one of the farms (herd B), these concentrations remained stable throughout the study period. In addition, the concentration of these microminerals fell within the reference intervals in both management types.

Nutrition & Metabolic Diseases

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Effect Of Particle Size Level And Forage Level On Physical Efficiency Of Total Mixed Rations Of Dairy Cows

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Objectives: The present research was conducted in order to determine the effect of two particle sizes of forage (short, SF and long, LF) and two forage levels (high, 60% HF and low 45% LWF, of DM) upon physical efficiency of completely mixed diets (CMD) offered during 30 d to commercially managed dairy cows.

Materials and Methods: In order to determine the effect of the particle's size over the physical efficacy of the administrated ration to Holstein cow was used a hammered mill which counted with two screens to obtain shredded alfalfa with two different particle's sizes. Both forage's levels were obtained by proportioning two forage's levels on diet, one with a 60:40 ratio, meanwhile the other with a dry mass ratio of 45:55. To determine the daily particle size distribution (PSD) and the physical efficiency of the diet (ef) a particle separator from the University of Pennsylvania (PSPS) was used. To estimate the physical efficiency of the neutral detergent matter (ef NDF) of the CMD the ef was multiplied by the NDF of the ration. The data was analysed by a 4x4 latin square with a factorial adjustment of 2x2.[P1]

[P1]

Results: The particle retention from the 19 mm screen was affected ($P < 0.001$) only by the particle size of the CMD. Meanwhile, the level of inclusion of the forage had an effect ($P < 0.001$) upon the amount of particles retained on the 8 mm screen. The amount of particles distributed

on de 1.18 mm screen was affected ($P < 0.001$) by the particle size as well as by the level of forage on the ration. On the other hand, the particle size had a significant effect ($P < 0.001$) upon the rations ef₂, having a larger amount on FL rations than SF (0.494 vs 0.474, respectively). The level of forage on the ration also affected the ef₂, finding a bigger amount on rations with LWF. On the ef₃, effect ($P < 0.001$) was shown on SF rations with larger amounts than LF (0.847 vs 0.835, respectively). The ef₂ NDF was not affected either by particle size or level of alfalfa inclusion. Both forage particle size and level of alfalfa inclusion affected ($P < 0.001$) the ef₃ NDF of the CMD.

Conclusions: In conclusion, the level of inclusion and particle size of alfalfa allows that a larger amount of feed is retained on the 19 mm screen but the amount of forage on the ration allows a greater physical efficiency of the NDF of cow's rations.

Nutrition & Metabolic Diseases

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In situ ruminal biohydrogenation of fatty acids of whole cottonseed affected with vitamin E or monensin

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Objectives: The aim of this study was to compare the kinetics of ruminal disappearance of unsaturated Fatty acids of ground whole cottonseed (WCS) affected with vitamin E or monensin using in situ nylon bags methode.

Materials and Methods: Two fistulated rams with the age of 15–20 months and 37 kg live weight were housed in individual pens for in situ trial. Animals were fed a completely mixed ration on maintenance level. The sheep were fed twice a day at 08:00 am and 16:00 pm. Seven days were assigned to adapt animals to the diet. There were 3 periods including 1) Whole cotton seed without additive, 2) WCS with Vitamin E (500 IU per kg DM) and 3) WCS with Monensin (24 ppm per kg DM per day). The times of incubation were 0, 2, 4, 8 and 24 h. Fatty acid methyl esters of oilseed samples were prepared using direct fatty acid methyl ester synthesis method. For GC–MS analysis, an Agilent 6890 gas chromatography with a 30m to 0.25mm HP-5MS capillary column coupled with an Agilent 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA) operating in EI mode at 70 eV was used. Proportions of individual fatty acids in bags were analysed for ground WCS, using the general linear model of SAS Institute.

Results: The results show that unsaturated Fatty acid (%) for treatments (1. WCS, 2. WCS +vitamin E and 3. WCS + monensin) at 2 h incubation were 34.26, 38.93 and 25.59 percent respectively, and treatment 2 numerically had the highest USFA content at time 2 h incubation. After 24 h ruminally incubation, treatment WCS + Vitamin E had highest USFA content among treatment (27.59%) and treatment WCS + monensin had the lowest USFA content (11.20%). The rate of Biohydrogenation for treatment 1, 2 and 3 were 9.55, 10.43 and 13.80 % per h, respectively.

Conclusions: Adding vitamin E reduced ruminally biohydrogenation of USFA and adding monensin increased ruminally biohydrogenation of USFA. Vitamin E could minimize the formation of trans-10 isomers in the rumen.

Nutrition & Metabolic Diseases

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Changes in colostrum composition in dependence on the time of parturition

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Objectives: The objective of our work was to determine the dynamics in the changes of concentration of selected parameters (Total protein, total immunoglobulin, immunoglobulin IgG1, lactoferrin, GGT, vitamins A and E) of bovine colostrum in dependence on the time of parturition.

Materials and Methods: Colostrum and milk samples were collected from the total yield from 10 cows of Holstein breed with the average performance of 10,850 kg of milk per standard lactation in the following periods.

The first sample was collected 1-2 hours after parturition (collection 1)
 The second sample was collected 6-12 hours after parturition (collection 2)
 The third sample was collected 32-48 hours after parturition (collection 3)
 The fourth sample was collected 60-90 hours after parturition (collection 4)

Colostrum samples were deep-frozen and kept at the temperature of 70°C until the time of analyses.

Total protein, total immunoglobulin concentration, immunoglobulin IgG1, lactoferrin, gamma-glutamyltransferase, vitamins A and E were determined in the samples of colostrum and milk.

Vitamins were established in whole colostrum using the method of liquid chromatography, other specified endpoints were determined in the treated colostrum by repeated centrifugation in the so-called cell-free and casein-free colostrum.

Total protein was determined colorimetrically on a Cobas Mira automated analyser using the 12751 P. f. Bio Vendor kit. Immunoglobulins (Ig) were determined by electrophoresis, immunoglobulins IgG1 were determined by radial immunodiffusion, lactoferrin, vitamins A and E were determined by liquid chromatography. The results were statistically evaluated. Mean values (X) and standard deviation (SD) were determined.

Results: Composition of colostrum changes after parturition significantly. Colostrum obtained from the first yield, in our case within 1-2 hours after parturition, had the highest concentration of all monitored parameters (total protein 129.04 g/l, IgG1 16.71 g/l, lactoferrin 1438.36 mg/l, GMT 2180.2 uka/T/L, VIT. A 17.91 UMOL/L, VIT. E 17.04 umol/L. Compared with the second milk yield (6-12 hours after parturition) there was a decrease in the concentrations of most parameters except vitamins A and E which remained at approximately the same level. The largest decrease was recorded in the value of IgG1 where the initial concentration 16.71 g/l dropped to 8.84 g/l. The relatively smallest decrease in the concentration between the first and second yield occurred in lactoferrin. From the third yield, i. e. after 32-48 hours, the concentrations of the monitored parameters in colostrum decreased rapidly, namely in total protein to 36.22g/l, IgG 3.33 g/l, lactoferrin to 601.28 mg/l, vit. A to 8.25umol/l and vitamin E to 7.44 umol/l.

Conclusions: The composition of colostrum in the period after calving changes significantly. The best quality colostrum comes from the first yield obtained immediately after parturition. The first colostrum has a high concentration of protein, with the highest proportion of immunoglobulins. It also has a high content of fat, minerals and vitamins. Other biologically active components are present in colostrum in concentrations higher than in milk. The concentrations of the monitored parameters reduced significantly in time after parturition.

Comments: The paper is based on the IGA VFU Brno project

Nutrition & Metabolic Diseases

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Addition Of Molasses And Corn Steep Liquor As Economical Sources To Enhance The Fungal Biomass Production Of Wheat Straw By Arachniotus Sp.

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Objectives: The study was planned to see the additive effect of various industrial residues including molasses (0, 0.2, 0.4, 0.6, 0.8 mL), rice polishing (0, 0.2, 0.4, 0.6, 0.8 gm) and corn steep liquor (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL) for bioprocessing of 5gm wheat straw (WS) through solid state fermentation (SSF) using Arachniotus sp. as fermenting agent.

Materials and Methods: Wheat straw (5g) used as solid state fermentation substrate was added into duplicate erlenmeyer flasks and adjusted at a substrate to water ratio of 1:2 on 4th day of incubation at 28°C and different salts concentration MgSO₄·7H₂O (0.050%), CaCl₂ (0.075 %), KH₂PO₄ (0.150%) and urea (0.15%) in growth medium. Inoculum (1 mL) of spore suspension (10⁶ to 10⁷ spores/mL) of Arachniotus sp. was added in the growth medium of each flask to obtain the maximum fungal protein. Crude protein contents of samples were tested according to the procedures of AOAC, 2006.

Results: A linear significant progressive increase in CP contents (p<0.001) was observed with increasing levels of C:N ratio. i.e. 0:1 (3.94%), 5:1 (3.99%), 10:1 (4.38%), 15:1 (4.65%), 20:1 (4.70%), 25:1 (4.81%) and decreased at 30:1 (4.59%) C:N ratio. Biomass CP contents (4.92%) were higher at 0.4mL level of molasses during SSF of wheat straw with Arachniotus sp., while the level decreased on further addition of molasses from 0.6mL (4.65%) to 0.8mL. Hence maximum fungal protein contents (13.40%) were found by cumulating 0.4mL molasses, 0.8gm rice polishing and 3.0mL corn steep liquor in treated wheat straw at a setting time of 48h for molasses and corn steep liquor in Solid state fermentation.

Conclusions: It was concluded that growth rate of Arachniotus sp. was greatly improved by addition of our selected amounts of carbohydrate and nitrogen sources. Hence, our current observations highly endorse the use of these economical by-products for cheap biomass production.

Nutrition & Metabolic Diseases

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Alterations in energetic metabolism in dairy cows during the periparturient period

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Objectives: The aim of this study was to evaluate dynamics of selected parameters of energy profil in dairy cows of the Slovak Pied Cattle from 3 weeks ante partum (a.p.) to 9 weeks post partum (p.p.).

Significant differences were found in average values of non-esterified fatty acids (NEFA), total lipids (TL), total cholesterol (TCH) ($P < 0.001$) and β -hydroxybutyrate (BHB) ($P < 0.05$).

Materials and Methods: Monitored parameters of energy profile – glucose, NEFA, BHB, triacylglycerols (TG), TL and TCH were evaluated in blood serum of dairy cows ($n = 15$, aged 3–5 years) of Slovak Pied Cattle. Blood samples were taken 3 weeks (wk) before parturition (3 wk a.p.), 1 week before parturition (1 wk a.p.), 1 week after parturition (1 wk p.p.), 3 wk p.p., 6 wk p.p., 9 wk p.p. by direct puncture of v. jugularis. Mean production age was 2.5 lactation. Milk yield during the previous lactation was 6668.5 kg of milk. The animals were fed a total mixed ration (TMR) twice daily and had free access to drinking water. The concentrations of NEFA (Randox, UK) and TL (Spinreact, Spain) were assessed by spectrophotometric method - Specord 210 Plus (Analytik Jena, Germany). The concentrations of BHB, glucose, TCH and TG were determined using commercial diagnostic kits (Randox, UK) on automatic biochemical analyser Alizé (Lisabio, France). Statistical analyses were done with the GraphPad Prism 3.0 software.

Results: Circulating concentrations of NEFA and BHB measure the success of adaptation to NEB and prepartum rise in NEFA suggested that cows were already in NEB at this time. The highest concentration of NEFA was found 1 wk p.p. (1.69 ± 0.55 mmol/l). Significantly lower values were observed 3 wk a.p. and 6, 9 wk p.p. compared to 1 wk a.p. and 1 wk p.p. ($P < 0.001$, $P < 0.05$). Decreased concentrations of NEFA in dairy cows 9 wk p.p. indicate that NEB was gradually corrected by reducing lipomobilization. The serum BHB concentration is indicator of energy metabolism disruptions which is more sensitive than glycaemia and which fluctuates in parallel to lipomobilization. The concentrations of BHB increased from 0.40 ± 0.12 to 0.83 ± 0.89 mmol/l from 3 wk a.p. to 3 wk p.p. ($P < 0.05$) then gradually decreased. The lowest concentration of glucose was detected 1 week p.p. in the context of the sudden activity of the mammary gland and the increased lactose synthesis. Serum TL concentration increased significantly p.p. compared with a.p. ($P < 0.001$). In TCH concentrations were observed significantly lower values in the cows a.p. and in the early postparturient period than were determined p.p. ($P < 0.001$ and $P < 0.01$, respectively). The highest concentration of TG was recorded 6 wk p.p., during a.p. were observed a decline of values. Concentrations of TCH and TG were affected by the physiological status, both showed substantial increases during p.p. Probably because, during the puerperal period, there is an increase in the demands for regulatory mechanism, responsible for all the processes involved with milking.

Conclusions: In conclusion, our results demonstrate that variables of parameters of energy profile as NEFA, BHB and glucose changed throughout the time around calving, which suggests that they have a physiological role in the dairy cow's energy metabolism. It can be concluded that, cows were in adequate energy status during monitored period and these results confirm the ability for adaptation in different physiological stages of lactation.

Nutrition & Metabolic Diseases

P01-001-077

Climate change and occurrence bovine reproductive conditions in the North West Province, South Africa.

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Objectives: Climatic changes induce with reduced rain and less grazing pasture for animals. This results in the occurrence of several cases of bovine reproductive conditions. The aim of this study was to evaluate the nutritional and haematological parameters in different reproductive conditions recorded in the Animal Health Hospital, North West University between June 2012 and June 2015.

Materials and Methods: A total of 108 cases of reproductive conditions such as downer cow syndrome ($n=13$), dystocia ($n=14$), retained placenta ($n=13$), vaginal prolapse ($n=9$), abortion ($n=28$) and those from cow in the final trimester of pregnancy ($n=31$) were recorded and blood samples collected together with other management information obtained from the farmer as well as the animal general clinical presentation. Blood samples were screened for brucellosis and serum analysed for blood biochemistry parameters. Structured questionnaires were also issued to farmers during farm visits and at community outreaches organized by North West University Animal Health Department. Data were analysed in SAS (version 20) using the analysis of variances techniques (ANOVA).

Results: The results were expressed as means \pm SEM. Dystocia was found to have the highest frequency (26.2%) of prevalence, followed by abortion and retained placenta with equal proportions of 23.1%, then downer cow syndrome (20%). The survey showed that significant differences were only observed in cow age ($P < 0.0001$) and breed types ($P = 0.0245$). The biochemical metabolites in this study all showed significant differences ($P < 0.05$) in breed types. It was noted that 53.6% of aborting cows, downer cows (61.5%), dystocia (50%) and vaginal prolapses (77.8%) tested positive for Brucellosis.

Conclusions: Recent drought caused by climate change and leading to poor grazing pasture due to low rain fall, reproductive diseases such as brucellosis, poor nutrition, lack of supplementation and poor management are the main cause of the occurrence of reproductive conditions reported in this study.

Nutrition & Metabolic Diseases

P01-001-078

Evaluation Of The Application Of Viusid Vet On The Bio Productive Indicators Of Milking Cows.

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Objectives: In Cuba as a third world country, the development of dairy cattle plays a very important role as each day increases the consumption of milk and its derivatives, It is these high demand for the population. The objective of this work is evaluate the influence of the supply of VIUSID vet on the bio productive behavior of milking Holstein cows

Materials and Methods: The work was performed in 16 and 17 dairy genetics Farm "Two Rivers" in the province of Sancti Spiritus, Cuba. Forty Holstein milk cows were used. In each unit, 20 cows were taken, which were divided in 2 homogeneous groups, considering the production of milk, feeding time, and body condition. The animals were carefully marked. The regime of feeding and management was the same for all animals.

The treatment used in each unit was as follows: group I. he was subject to the existing system of exploitation. Its composition was

10 animals and II was administered mixed with food during milking in proportion VIUSID 50 g of powder per animal vet for 30 days. The product is administered for 30 days but the study is extended for 50 days.

The variables studied were: milk production, presentation of jealousy and Incidence of endometritis and other reproductive disorders

Results: At the beginning of the experiment, but no significant differences, the mean values of milk production in the control group are slightly higher than the treated group, however, was observed that, starting when the cows had been eating the VIUSID vet for 21 days there was a significant increase in the production of milk in the treated animals (22.5%) in regards to the control group, said effect remained at 36 days (29.9%) and 49 days (26.6%) after the trial started, demonstrating the persistence of the effects of the product after its consumption is finished considering that it is only provided to the cows for 30 days. Also, 60% of the cows in the treated group had a heat cycle, while only 45% of the cows in the control group had it. On the other hand, in the control group, 10% of the cows presented endometritis, while in the treated group none of the cows were affected by this pathology.

The better behavior of the animals can be given by the effect of product composition, which may be supplementing important elements necessary for the growth and development functions of these animals, especially if one considers that the molecular activation is performed at the same effects that it incorporates no components by themselves

Conclusions: The supply of VIUSID vet in the studied dose significantly improves the bio productive behavior of the milking Holstein cows

There is a tendency to improve reproductive rates and reduce the incidence of endometritis in cows consuming the product

Nutrition & Metabolic Diseases

P01-001-079

Selected variables of protein profile and their relation to indicators of lipid mobilization in dairy cows after calving

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Objectives: In dairy cows, the metabolic changes occurring in the period around calving may influence the incidence of peripartum diseases. The mechanism of association between metabolic changes and inflammatory diseases around calving is not entirely clear, but may be mediated through diminished immune-cell functions. The aim of this study was to determine the concentrations of the main indicators of lipomobilization and selected variables of protein profile in dairy cows after calving, including immunoglobulins and acute phase proteins, as well as to evaluate the relationships between the altered lipid metabolism and changes in protein profile.

Materials and Methods: Into the evaluation we included fifty four dairy cows of a Slovak spotted breed, low-land black spotted breed and their crossbreeds in the period from one to two weeks after parturition. The animals were housed in free-stalls, and fed twice a day diets for lactating cows. The evaluated cows were without health disorders during the observation. Blood samples were analyzed for non-esterified fatty

acids (NEFA), beta-hydroxybutyrate (BHBI), total proteins, albumin, immunoglobulin G (IgG), haptoglobin (Hp) and serum amyloid A (SAA). The concentrations of NEFA, BHB, total proteins and albumin were assessed using commercial diagnostic kits (Randox, United Kingdom). The concentrations of IgG and SAA were analysed by enzyme linked immunosorbent assay (ELISA) using commercially available diagnostic kits (Cusabio, China, Tridelata Development, Ireland, respectively). Haptoglobin was assessed using commercial colorimetric kits (Tridelata Development, Ireland) in microplates. The obtained results from evaluated cows were divided into two groups according to the measured concentrations of NEFA: Group A (n = 34) – cows with serum concentrations of NEFA below 0.35 mmol/l and Group B (n = 20) – cows with serum concentrations of NEFA above 0.35 mmol/l. Unpaired Student's test was used to evaluate the significance of differences in means between the groups of cows. Relationships between the concentrations of the evaluated variables in the monitored cows were calculated by linear regression and Pearson correlation test.

Results: In cows with higher concentrations of NEFA (Group B) we found significantly lower mean serum concentrations of total proteins, albumin and IgG than in cows with lower serum NEFA concentrations (Group A). On the other hand, cows with higher values of NEFA showed significantly higher mean concentrations of haptoglobin and serum amyloid A. Trend of significantly higher values in cows with higher NEFA concentrations was found also in the concentrations of BHB. The concentrations of NEFA significantly negatively correlated with the values of IgG (R=-0.638, P<0.001). Significant negative correlations was found also between the concentrations of NEFA and total serum proteins (R=-0.436, P<0.001), as well as between NEFA and albumin (R=-.395, P<0.01). Between the concentrations of NEFA and haptoglobin, as well as NEFA and serum amyloid A significant positive correlations were found (R=0.588, P<0.001, and R=0.811, P<0.001, respectively).

Conclusions: This study indicates relationships between the concentrations of NEFA and variables of protein profile in cows after parturition. The study demonstrates that metabolic changes associated with energy imbalance and fat mobilization may be related to alterations in protein metabolism. These data contribute to the better understanding of the metabolic changes occurring in dairy cows after calving. However, further studies are needed to investigate in detail the association of changes in protein profile with alterations in lipid metabolism.

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Nutrition & Metabolic Diseases

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Edible oil intoxication in natural cases of crossbred cows – a clinical study

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Objectives: Edible oils particularly peanut, sesame and coconut oil are part of stable food of India. Peanut oil and sesame oil contains a high proportion of unsaturated fatty acids. Coconut oil contains high level of saturated fatty acids. Unsaturated fatty acids have more potent antimicrobial effects and promote greater inhibition of ruminal fermentation than saturated fatty acids. Hence the accidental consumption of excess quantity of edible oils produces clinical illness in cattle. This may be the

first report in edible oil intoxication. This research describes the clinical signs, treatment and clinical outcome in five oil intoxicated cases.

Materials and Methods: Research was carried out at Teaching Veterinary Clinical Complex(TVCC), Veterinary College and Research Institute, Orathanadu – 614 625, India during March 2014 to September 2015. Five animals were reported with history of Peanut (3 animals), Sesame (1 animal) and Coconut oil (1 animal) intoxication and admitted in large animal outpatient unit of TVCC mostly at summer. Three animals bearing number 2141/14, 5916/14 and 4721/14 consumed 4, 3 and 6 liters of peanut oil respectively. Others 469/15 consumed nine liters of sesame oil, and 3080/15 consumed three liters of coconut oil. The rumen fluid examination revealed no protozoal activity in three peanut oil intoxicated animals. Two animal's (469/15 & 3080/15) rumen fluid had oil bubbles encapsulated with protozoa. The complete blood count was carried out in automated hematology analyzer (Vet scan HM5, Abaxis). All the animals were treated with polyionic fluids like multiple electrolyte solution, ringer's lactate, 7.5% sodium bicarbonate, ruminotronics and B-vitamins. In three animals (2141/14, 5916/14 and 469/15) rumen gut transplantation was attempted. Sodium bicarbonate was administered orally @ 100-200 gms as total dose according to the clinical recovery. Antibiotics like Strepto-penicillin was administered with commercial preparations of ruminal antacids (Powder Bufzone*). Two animals (2141/14 & 5916/14) have intestinal mucosal shred in dung was treated with light kaolin. Owners were encouraged to administer oral rehydration therapy with polyionic solutions.

Results: Animals particularly cattle returning from common grazing area with full of thirsty (temperature reaches 40°C in hot summer) tense towards drinking of liquid. This situation conjugates this research work as edible oil intoxication. The clinical signs in peanut oil intoxication are anorexia from day one onwards, ruminal atony, cessation of rumination, dehydration, diarrhea initially which turned to semi solid in consistency with mucus shreds, perineal pasting, heavy fetid smell of dung, dull and depressed. After four days a (2141/14) animal developed cud dropping. Another animal (4721/14) had ataxia and tremor in fore quarter muscles. Sesame oil intoxicated animal had anorexia, dehydration, severely congested mucus membranes, ruminal atony, semisolid oil mixed dung, staggering gait from fifth day onwards and after 7days animal went for recumbence, inanition and cachexia. Coconut intoxicated animal had transient anorexia followed by varying degree of appetite, mucus and oil coated dung and latter diarrhea. All the animals had neutrophilia and leukocytosis. Peanut oil intoxicated bullock (2141/14) recovered completely after 15 days of therapy. No clinical recovery was noticed inspite of intense therapy after a week in a heifer (5916/14). It was sent to emergency slaughter. Another peanut intoxicated animal died on the next day of intoxication which had ataxia (4721/14). Sesame oil intoxicated animal died after 19 days. Owner discontinued therapy after 13 days because of frustration. Complete recovery noticed after 4 days of therapy in coconut oil intoxication.

Conclusions: This research concludes severity of illness was more in peanut and sesame oil intoxication. The major clinical signs were anorexia, cessation of rumination, ruminal atony, watery to semi solid diarrhea with mucosal shreds in the dung, dehydration and inanition. No protozoal activity was observed in rumen fluid of peanut oil intoxicated animals. The unsaturated fatty acids might be toxic to the mucosal epithelial cell membrane resulting in the denudation of intestinal epithelium. Adequate fluid and electrolytes, sodium bicarbonate, gut transplantation and broad spectrum antibiotics will help in clinical recovery of oil intoxicated animals.

Parasitology

P04-004-059

Migration of *Fasciola hepatica* Newly Excysted Juveniles Is Inhibited by High- and Oligomannose-Type N-Glycan Binding Lectins In Vitro

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Objectives: *Fasciola hepatica* infection has major economic impacts on cattle production worldwide. In the host, following ingestion of metacercariae, the Newly Excysted Juveniles (NEJ) hatch in the small intestine, attach to the enterocytes and migrate through the intestinal wall until they reach the peritoneal cavity. Although the importance of selected molecules in NEJ invasion is known, many relevant proteins secreted by the NEJ are glycosylated, and the role of host and parasite-derived glycans in this process is not. Therefore, the aim of this work is to assess the differences in the migration rate of the NEJ after blocking different glycan subsets.

Materials and Methods: *F. hepatica* NEJ were hatched in vitro and incubated for 90 min with one of eight biotinylated lectins (ConA, GNL, PNA, Jacalin, UEA, LCA, PSA and WGA) that were chosen for their carbohydrate-binding affinities and ability to bind to glycoproteins in the NEJ tegument (NEJTeg) and soma (NEJSom). In parallel, distal jejunum from a euthanized rat was mounted in chambers in an in vitro system optimised for measuring the migration of NEJ across the intestine. Tissues in the chambers were incubated with lectin-incubated NEJ, and parasite migration through the intestine measured every 30 min up until 150 min. In addition, small batches of lectin-incubated NEJ were fixed in formalin solution at the beginning of the experiment, (T0) and at the end (T150). Fixed NEJ were embedded in paraffin for histological analysis to confirm positive lectin-glycan binding. In a second experiment, rat jejunal tissues were incubated for 60 min with the lectins WGA, GSL-I or UEA. Lectins were chosen according to their ability to bind to intestinal tissue. The lectin-incubated chambers were then incubated with *F. hepatica* NEJs.

Results: Incubation of NEJ with lectins binding to high and oligomannose-type N-glycans (ConA and GNL) significantly inhibited NEJ migration from 90 min till the end of the experiment. Histochemistry performed on sections of lectin-incubated NEJ showed that ConA and GNL did not bind to NEJSom but bound to the NEJTeg and the oral and ventral suckers from T0 to T150 min. Migration rates of the rest of lectin-incubated NEJ were not significantly different from those of the untreated group. WGA-, GSL-I- and UEA-incubated jejunal tissues did not affect the NEJ migration trend.

Conclusions: We concluded that the blockage of high and oligomannose-type N-glycans on *F. hepatica* NEJ with lectins significantly inhibited the migration of the juvenile flukes through the intestine in vitro. The development of drugs or vaccines that could mimic these inhibitory effects of ConA and GNL lectins could provide potential novel tools for the control of fasciolosis.

Parasitology

P04-004-061

In vitro and in vivo evaluation of the herbal complex against trichostrongylid nematodes of sheep

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Objectives: Scientific validation of a herbal complex (HC), based on aqueous extracts of *Azadirachta indica* leaves, *Calotropis procera* flowers and *Trachyspermum ammi* seeds which have been used empirically as anthelmintic against trichostrongylid nematodes of sheep.

Materials and Methods: In vitro, eggs and adult *H. contortus* were exposed to different concentrations of HC following the standard procedures of egg hatch test (EHT; 50 to 0.024414 mg ml⁻¹) and adult motility assay (AMA; 200-0.1953125mg ml⁻¹), respectively. The reference drugs used in the study were oxfendazole (0.0056704 to 0.0000027 mg ml⁻¹) and levamisole (1.50 mg ml⁻¹) for EHT and AMA, respectively. In vivo, pre-and post-treatment (4 mg, 2 mg and 500 µg kg⁻¹ body weight) fecal egg counts were determined following standard fecal egg count reduction test in sheep naturally parasitized with mixed species of gastrointestinal nematodes.

Results: In EHT, LC₅₀ values of HC and oxfendazole (reference drug) were 275.1 and 0.016 µg ml⁻¹, respectively. In AMA, 100% mortality of *H. contortus* was observed 6 hrs post- exposure to 3.125-200 mg ml⁻¹ concentrations of HC and 2 hrs post-exposure to levamisole. In vivo, maximum (96.2%) fecal egg count (EPG) reduction was recorded in sheep treated with HC @ 4 mg kg⁻¹ body weight; whereas, 89.3% reduction in EPG was recorded in sheep treated with levamisole @ 7.5 mg kg⁻¹ body weight. The results of higher doses of HC were comparable with the reference drugs.

Conclusions: A graded dose response of HC in all the tests used in present study exhibits anthelmintic activities of the HC and justifying its traditional use as anthelmintic. Further, this study provides base-line information for future steps of drug development on commercial basis.

Parasitology

P04-004-063

Tolfenpyrad Insecticide Cattle Ear Tags: New Chemistry for Horn Fly *Haematobia irritans* control on cattle

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Objectives: The objective of these studies was to evaluate efficacy of a tolfenpyrad cattle ear tag formulation (Tolfenpro™) for horn fly (*Haematobia irritans*) control on cattle. Tolfenpyrad, a new insecticidal active ingredient, belongs to the “mitochondrial electron transport inhibitor” (METI) mode of action (MOA) group [Insecticide Resistance Action Committee (IRAC) Group 21]. It represents a novel MOA for

ectoparasite control on cattle, and new rotational option for insecticide resistance management and horn fly control.

Materials and Methods: Five field studies were conducted in Missouri, Oklahoma and Texas (USA) in 2012-2013. Studies followed similar protocols, with each using five groups of cattle with 10 or 15 animals per group. In each study, one group served as a negative control (no ear tag treatment), and the other four groups received ear tag treatments. The tolfenpyrad ear tag formulation was compared to commercial ear tags containing active ingredients from three other IRAC MOA groups: avermectins (IRAC Group 6; XP 820™ - 8% abamectin + 20% piperonyl butoxide [PBO]); organophosphates (IRAC Group 1B; Corathon® - 15% coumaphos + 35% diazinon); and two pyrethroids (IRAC Group 3; PYthon™ Magnum® - 10% zeta-cypermethrin + 20% PBO and CyLence Ultra® - 8% beta-cyfluthrin + 20% PBO).

Following pretreatment fly counts animals were treated with the ear tag formulations, and horn flies were counted at approximately weekly intervals for up to 21 weeks post-treatment. Study duration varied with location and year; all studies were terminated when horn fly counts on treated animals were equal to or greater than counts on control animals.

Data were evaluated using two approaches. First, the weekly post-treatment fly counts were used to determine each ear tag's duration of efficacy, defined as the number of weeks for which the ear tag treatments reduced horn fly counts $\geq 90\%$, compared to the untreated controls. Second, the cumulative total numbers of horn flies counted on each animal during each study were analyzed to determine the overall reduction in fly pressure resulting from the ear tag treatments.

Results: The duration of efficacy ($\geq 90\%$ reduction in weekly average horn fly counts) varied substantially within and between studies. The average (range) numbers of weeks of efficacy for the five ear tag formulations were: tolfenpyrad 14 (8 - 21); abamectin + PBO 13 (9 - 21); coumaphos + diazinon 13 (9 - 16); zeta-cypermethrin + PBO 9 (5 - 17); and beta-cyfluthrin + PBO 8 (0 - 19).

All five ear tag formulations significantly reduced overall horn fly pressure ($p < 0.05$), compared to the untreated control animals. Across all five studies the average (range) percent reductions in cumulative horn fly numbers were: tolfenpyrad 79% (67 - 91%); abamectin + PBO 79% (63 - 90%); coumaphos + diazinon 73% (63 - 83%); beta-cyfluthrin + PBO 66% (56 - 85%); and zeta-cypermethrin + PBO 64% (31 - 81%).

Conclusions: Tolfenpyrad efficacy against *H. irritans* was comparable or superior to avermectin, organophosphate and pyrethroid products. These results indicated that tolfenpyrad's MOA represented a significant addition to other chemistries available for horn fly control and resistance management. Furthermore, tolfenpyrad has not been used extensively in crop protection, thereby reducing the risk of inadvertent horn fly exposure.

Bayer has obtained U.S. Environmental Protection Agency registration for this tolfenpyrad ear tag (Tolfenpro) for horn fly control on beef and dairy cattle, and is evaluating development potential in additional countries.

Parasitology

P04-004-064

Association between rumen fluke and liver fluke prevalence in ruminants in Ireland

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Objectives: Diagnostic laboratories in Ireland and the UK have reported an increase in the presence of rumen fluke (*Calicophoron daubneyi*) eggs in faecal samples submitted from both cattle and sheep starting in the late 2000's. As *C. daubneyi* is believed to share the intermediate snail hosts, *Galba truncatula* and *Lymnaea glabra*, with the liver fluke (*Fasciola hepatica*), the two fluke species are expected to co-occur. This epidemiological study was undertaken to investigate whether co-infections in ruminants are correlated geographically and/or seasonally.

Materials and Methods: Information on the presence and absence of liver and rumen fluke eggs in bovine, ovine, equine and caprine diagnostic faecal samples during 2012, 2013 and 2014 was provided by ParaLabs Ltd, the diagnostic laboratories of the Veterinary School in UCD and the Regional Veterinary Laboratories in Ireland. Statistical analysis was conducted to investigate the seasonality of both parasites and any possible association between them. Additionally, infection rates in the various host species were compared. Finally, the geographical distribution of both parasites across Ireland was examined using Geographical Information System (GIS) tools.

Results: There was a moderate association between rumen fluke and liver fluke as co-infections of the two were widespread supporting the hypothesis of shared intermediate hosts. However, there were significant differences in the distribution of the two flukes in the various host species, most notably a higher infection rate of rumen fluke in cattle while prevalence for both parasites in sheep was similar. The seasonal distribution of the two parasites varied from year to year with annual peaks in winter or spring.

Conclusions: Differences in fluke distributions are likely to reflect drug use and treatment schedules commonly used in the various host spp, but may also be due to differences in the availability of intermediate snail hosts in areas. Variations in the seasonality of both diseases may be related to changes in climatic conditions from year to year and may influence the risk of infection overall. Considering the co-occurrence of the 2 fluke species it is important to determine whether one can exacerbate the clinical effects of the other.

Parasitology

P04-004-065

Outbreak of bovine trypanosomiasis due to inadequate pre-milking management in the state of Goiás, Brazil

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Objectives: Trypanosomiasis is one of the world's most important human and livestock disease. In this paper, we report an outbreak of bovine trypanosomiasis occurred in the month of September 2015 in Goiás State, Brazil, that involved an infection with *Trypanosoma vivax* in dairy cattle.

Materials and Methods: The outbreak reported here occurred on a dairy farm with 418 Girolando animals, in a semi-intensive production system. The farmer manager was interviewed in order to obtain information about health problems of the herd, movement of animals into and out

from the farm and others risk factors that might have contributed towards the spread of the disease. From a total of 418 cattle, 67 cows showing clinical signs were examined and blood samples were collected from the jugular vein for direct observation of fresh blood samples.

Results: As reported by the farm manager many of the cows were bought in others states (Sao Paulo e Minas Gerais) and a total of 40 cows died. The main factors involved in the spread of the disease were: the entry of animals without prior tests and quarantine and the cows received daily 5 IU of oxytocin before milking, to assist in milk let-down, which was injected on the cranial superficial epigastric vein, using the same needle and syringe in all animals. They never observed any large population of tabanids, which are the flies implicated as the main vectors of *T. vivax* in South America. *Haematobia irritans* and *Stomoxys calcitrans* weren't detected during all the visits to the farm. From a total of 418 cattle, 67 (16%) showed signs of anemia, lethargy, weakness, general weight loss, hyperthermia, anorexia, reduction in milk production, abortion and retained placenta. The protozoan was observed microscopically in 36 (8.61%). No other blood parasites, such as *Babesia* sp. and *Anaplasma marginale*, were observed in the microscopic exam.

Conclusions: Iatrogenic transmission through inappropriate application of intravenous medication in the pre-milking management, associated with the acquisition of animals of unknown origin, may be grounds for rapid spread of bovine trypanosomiasis in a dairy herd.

Parasitology

P04-004-066

Identification of *Sarcocystis tenella* (Protozoa: Apicomplexa) in Korean Native Goat from Korea

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Objectives: *Sarcocystis* species are common parasites with a wide range of hosts and obligatorily intracellular protozoa. There are three species of *Sarcocystis* in domestic goats: *S. capracanis*, *S. hircicanis* and *S. capraefelis* (syn. *S. moulei*). *S. capracanis* and *S. hircicanis* produce microscopic sarcocysts while *S. capraefelis* produces macroscopic cysts. *S. capracanis* is the most pathogenic species in goats, causing fever, weakness, anorexia, weight loss, tremors, irritability, abortion and death. In this study, we report the natural infection of *S. tenella* in Korean native goat for the first time.

Materials and Methods: A total of 103 cardiac muscle samples of Korean native goats, *Capra hircus coreanae*, were obtained from January to August, 2014 in slaughterhouse located in Wonju city, the Republic of Korea. The specimens were examined histologically analyzed by light microscopy (LM), transmission electron microscopy (TEM), and complete 18S rRNA gene product obtained by polymerase

chain reaction (PCR) was sequenced and phylogenetically analyzed. Genomic DNA was next extracted from frozen positive samples identified by light microscopic method. Genomic DNA extraction was carried out using DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany) according to manufacturer's instruction. The PCR products were next visualized via electrophoresis on 1.2% agarose gel with SiZer™-100 DNA marker (Intron, Seongnam, Korea) and purified using Nucleospin® Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany) for sequencing.

Results: To investigate the prevalence of *Sarcocystis* sp. in the Korean native goat, the heart tissues were analyzed using H&E staining. Of the 103 goats, 3 goats were diagnosed as sarcocysts positive, and they are identified with *S. tenella* using H&E staining, electron microscopic analysis and genomic DNA sequencing. The incidence of *Sarcocystis* infection is low (2.91%) in the slaughtered goat. Using a transmission electron microscopy, the sarcocysts are identified with *S. tenella*. Moreover, DNA and phylogenetic analysis confirmed our identification of *S. tenella* using 18S rRNA gene sequencing.

Conclusions: Of the 103 goats, 3 (2.91%) were diagnosed as positive for *S. tenella* by light, electron microscopic and molecular examination. The histopathological study showed a low frequency of microscopic *Sarcocystis* infection in slaughtered goats. In transmission electron microscopy, the sarcocysts were confirmed as *S. tenella*. Further DNA sequencing and phylogenetic analysis support our identification of *S. tenella* with the sequence identity of 18s rRNA between the experimental sequence and *S. tenella* with 100%. This is a first record of *S. tenella* in Korean native goat from Korea.

Parasitology

P04-004-067

Targeted selective treatment of gastrointestinal nematodes based on live weight gain variations in first-season grazing dairy calves in Ireland

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Objectives: As anthelmintic resistance is increasingly evident in cattle populations, targeted selective treatment (TST) strategies are aimed at ensuring a more responsible use of anthelmintic molecules. The aim of the current study was to investigate the ability of TST in first-season grazing Irish dairy calves to 1) limit gastrointestinal nematode infection levels, reduce anthelmintic use and maintain animal performance, and 2) be practical and resource efficient (i.e. farmer-friendly), when implemented under Irish environmental conditions and grazing management practices.

Materials and Methods: The experiment was conducted at Teagasc, Moorepark (Co. Cork, Ireland). The study period began 6th May 2015 for a period of three months. Calves were rotationally grazed over the course of the experiment. Prior to treatment, 110 female dairy calves born between 22nd January and 18th February 2015 were randomised by breed, date of birth, body weight at trial initiation and live weight gain (LWG) to one of three anthelmintic treatment strategies; no treatment (NT, n=10), group treatment (GT, n=50) and targeted selective treatment (TST, n=50). The NT group received no anthelmintic treatment over the course of the study to allow determination of the overall nematode challenge. A prophylactic injection of long-acting moxidectin was

administered to the GT group on 6th May (Cydectin® 10% LA, Zoetis) as per routine nematode management on the farm. Treatment of TST calves was based on two LWG criteria and one health criterion. Live weight gain was assessed every second week and calf health was scored twice weekly (including faecal score). A TST calf (1) failing to reach a LWG threshold set at 20% below the LWG average of its grazing group, or (2) showing a LWG below 0.56 kg/day (i.e. 20% below an optimum of 0.70 kg/day), or (3) with diarrhoea (faeces score of 3/3 on two consecutive occasions) received anthelmintic treatment within one week. For monitoring purposes, faecal samples were collected every month for gastrointestinal nematode faecal egg count (FEC) determination. Descriptive data were collated and analysed in MS Excel (2010).

Results: Of the 50 calves in the TST group, 16, 4, 8, and 1 were treated within 10, 31, 46, and 65 days of trial start date, respectively. In all, therefore, 60% of the TST group required dosing. Those calves in the TST group that required treatment were dosed on the basis of failing to meet LWG criteria. No calf was treated on the basis of a poor faeces score. No difference in body weight at the end of the trial period (150 vs. 152 kg; $P=0.41$) or overall LWG over the duration of the trial (0.68 vs. 0.70 kg/day; $P=0.42$) was recorded between TST and GT groups. The worm challenge experienced over the course of the study was moderate, with a mean FEC of 158 eggs per gram (range 0 to 700 eggs per gram) in NT calves after 120 days of grazing. No difference in FEC was observed between TST and GT calves.

Conclusions: This study demonstrated that in Ireland, gastrointestinal nematode parasitism of first-season grazing dairy calves could be controlled using a TST strategy without any significant effect on calf performance. It must be stated, however, that only 40% of TST calves remained undosed. The economic benefit of reducing anthelmintic use was, however, far outweighed by the resources (labour, equipment, diagnostics) required to effectively implement a TST programme without compromising calf health. The practicality of implementing such an approach in grass-based systems such as exists in Ireland, therefore, is highly questionable currently.

Parasitology

P04-004-068

Prevalence of non-*Tritrichomonas foetus* trichomonads in preputial samples from Nebraska beef bulls

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Objectives: Bovine trichomoniasis is a venereal disease of cattle found throughout the world caused by *Tritrichomonas foetus*. Control programs rely on detection and removal of carrier bulls. Non-*T. foetus* trichomonads may lead to false positive microscopic examinations. False positive PCR results due to non-*T. foetus* trichomonads are less likely; however PCR inaccessibility and cost may prevent its use. The purpose of this study was to determine the prevalence of non-*T. foetus* trichomonads in the preputial samples from a herd of Nebraska beef bulls to allow diagnosticians to make informed interpretations of culture results.

Materials and Methods: Preputial specimens were collected from all bulls in a single herd with no history of trichomoniasis. The bulls were of various breeds and ranged in age from one to five years and were sexually rested for at least four months prior to sample collection using a

previously described dry pipette technique. All samples were inoculated into a commercially available plastic pouch containing culture medium immediately after collection and maintained at room temperature until placed in an incubator within four hours of collection. Inoculated pouches were incubated upright at 37°C for four days. Pouches were systematically examined daily for the presence of trichomonads by an experienced veterinarian using light microscopy starting 24 hours after collection and continuing through the fourth day after collection. Pouches were classified as positive if live protozoa of a size, morphology, and motility pattern consistent with trichomonads were seen on at least one of the five days. After the microscopic examination on the fourth day all culture positive pouches were submitted for gel PCR testing using *T. foetus* specific and pan trichomonad primers according to the laboratory's standard protocols.

Commercially available software was used to calculate the prevalence and 95% confidence interval of non-*T. foetus* positive samples.

Results: Three hundred and fifty-two bulls from one to five years of age were sampled and produced three culture positive, *T. foetus* PCR negative, pan trichomonad PCR positive samples resulting in an overall non-*T. foetus* prevalence of 0.85% (95% CI, 0.29% to 2.48%). All three positive samples were from yearling bulls resulting in a non-*T. foetus* prevalence of 2.0% (95% CI, 0.68% to 5.71%) for this age group of 150 bulls. All 202 bulls two years of age and older were culture negative resulting in a non-*T. foetus* prevalence of 0.0% (95% CI, 0.0% to 1.87%) for this age group.

Conclusions: The current study supports the assumption young bulls are more likely to harbor non-*T. foetus* trichomonads than older bulls. Although no older bulls were test positive in this study the 95% confidence interval indicates the true prevalence is less than that of yearling bulls, but not necessarily zero. Therefore, preputial samples tested for *T. foetus* by culture should be confirmed as truly *T. foetus* positive by other testing procedures as indicated by pretest factors such as herd and bull history, bull age, and the estimated prevalence of non-*T. foetus* trichomonads.

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Parasitology

P04-004-069

Generation of resistance reversion to Macroclastic Lactones in cattle throughout epidemiological and refugia principles

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Objectives: Nematodes that are not exposed to anthelmintic are known as refugia. Appropriate usage of refugia is reported to assist in slowing the rate of resistance development by diluting the genetic significance of the resistant worms surviving in animals after treatment.

The objective of this study was to determine if quantifiable, well-timed and appropriate dilution could quickly create an improvement in efficacy of the ML family and, if so, collect a dataset that could be used to improve our understanding of just how much refugia is enough to make meaningful changes in established (or developing) resistance levels in nematode populations.

Materials and Methods: A farm in Argetina was selected, based on it having a 2012 Fecal Egg Count Reduction Test (FECRT) result for Ivermectin (IVOMEC®, injection at 200 mcg/kg) against *C. oncophora* of 74.7%. A single paddock (65 hectares) on this farm was used for this study. The overall design was to inoculate ML susceptible *C. oncophora* into calves at a time of the year when larval survival on pasture was normally at its lowest (late Summer) and to then graze these cattle on a paddock with established resistance.

Eighty-two, 6-month old Aberdeen Angus heifer calves were treated with a Levamisole subcutaneous injection (Ripercol®, 7.5 mg/kg) and then 7 days later were inoculated orally with 20,000 L3 of *C. oncophora*. 14 days later, the oral challenge of L3 was repeated and the cattle were then placed on the test paddock (March 2013 - Autumn). Tracer calves were also placed in the paddock in Autumn and again later in Spring, with both groups being subsequently treated with Ivermectin (IVM) and subjected to post-treatment worm counts to determine Macrocytic Lactone (ML) efficacy. An IVM FECRT was also conducted on the 82 calves in November (late Spring). At monthly intervals feces from animals and pasture grass samples were collected. Rainfall levels at the farm were also recorded.

Ripercol® is a registered trademark of Zoetis. IVOMEC® is a registered trademark of Merial Inc.

Results: The efficacy of Ivermectin against *C. oncophora* in the Tracer Calves were 39.1% in Autumn and 75.7% in the following Spring (Worm Kill results). The FECRT performed on permanent grazing heifers at the end of the spring showed a clinical efficacy overall of 95% with *Cooperia* spp. being the single worm species identified in post-treatment coprocultures. The FECRT result for *C. oncophora* was 61% but with a day 0 *Cooperia* spp. count of 28 eggs per gram. The pasture contamination level at initial placement in March was 125 L3 per kg dry matter. Fecal cultures and herbage infectivity indicated that contamination with nematode eggs throughout the study was clearly dominated by *Cooperia* spp. (> 50%). The levels of infective larvae were very low during winter and from September onwards an increase was recorded up to reach a peak of 1486 L3/k.d.g. in November.

Conclusions: It was not expected that the efficacy of Ivermectin would return to >95% after just 1 year, especially after seeing the very low efficacy recorded in tracer calves in Autumn (39.1%). By the end of the year the worm kill result had well improved (75.7%). Despite a low level of herbage infectivity at beginning of the study (125 L3 per kg dry grass) it was possibly high enough (combined with concurrent rain events) to ensure that meaningful dilution of the resistant worm population was going to be difficult to quickly achieve. It is expected that repeating this process over the next few years will further improve the efficacy levels.

Parasitology

P04-004-070

In vitro and in vivo, acaricidal activity of a herbal formulation against cattle tick (*Rhipicephalus microplus*)

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Objectives: Evaluation of anti-tick activity of a herbal formulation (HF) based on aqueous extracts of *Azadirachta indica* and *Nicotiana tabacum*

leaves, *Calotropis procera* flowers and *Trachyspermum ammi* seeds against cattle tick *Rhipicephalus* (*Boophilus*) *microplus*.

Materials and Methods: Eggs, larvae and adults of *R. microplus* were subjected to various doses of HF (Deionized water). In vitro, 10 adult blood engorged female ticks were immersed in various dilutions of HF (Adult Immersion Test (AIT); 50 - 6.25 mg ml⁻¹) and 100 larvae exposed to HF (Larval Packet Test (LPT); 50 - 0.78125 mg ml⁻¹) through impregnated filter papers. In vivo, twelve calves (n=12; local breed) were infested with unfed larvae (14 days old) in ear bags (13 × 17 cm; 110-130 larvae/bag) tied on the base of ear pinnae. After 24 hrs of the larval attachments, three animals (replicates) for each suspension of HF (15%, 30% and 45%) were treated topically on the tick infested body parts for 6 consecutive days, twice-a-day.

Results: The HF demonstrated anti-tick activity by inhibiting the fecundity of the female ticks, hatching percentage of eggs, larval mortality and reduced tick intensity/infestation on animals. In vitro, lethal effects on egg laying (index of egg laying = 0.371404 ± 0.00435), hatching (22.35%) and 100% larval mortality were recorded at 50 mg ml⁻¹. In vivo, the HF reduced tick intensity significantly on the infested calves (18 detached out of 35) at 45% (w/w) suspension, applied topically on the infested skin.

Conclusions: A graded dose- and -time dependent response of the HF in this study, against all developmental stages of *R. microplus* indicates potential of the HF as a botanical acaricidal. Study of toxicity of the HF on exotic breeds of cattle, however, is recommended.

Parasitology

P04-004-071

Identification Of Enteropathogens From Murrah Buffalo Calves

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Objectives: The present study aims to assess the occurrence of endoparasites and rotavirus in fecal samples from a dairy property of Murrah buffalo calves from different ages, and assess the presence of rotavirus in those animals.

Materials and Methods: There were collected fecal samples from ninety one Murrah buffalo calves distributed in the following groups: G1: 0-30 days old (n=7); G2: 31-60 days old (n=14); G3: 61-90 days old (n=4); G4: 91-120 days old (n=6); G5: 121-150 days old (n=9); G6: 151-180 days old (n=9); G7: 181-210 days old (n=15); G8: 211-240 days old (n=15); G9: 241-270 days old (n=7); G10: 271-300 days old (n=5). All fecal samples were inspected to determine the absence or presence of diarrhea. The research was made in a dairy farm located in the city of Alambari located in the State of São Paulo, Brazil. The samples were collected directly from the rectum using plastic hand gloves and identified with the animal number and age, then they were preserved at 4°C until the analysis. The parasitological analysis were made according to the Gordon &

Whitlock modified technique and the identification of rotavirus by polyacrylamide gel electrophoresis (PAGE) technique following silver nitrate stain.

Results: Twenty calves of the total examined (21.98%) had diarrheal feces. Seventy eight of the ninety one samples (85.71%) were positive for *Eimeria* sp., with more prevalence in G2 and G6. Sixty six of the total samples (72.57%) were found positive for Strongylida, with only one positive animal in G2 and the higher infestations on the G5 (average value of 1,083 EPG count). Three samples were positive for *Moniezia* sp. (3.30%), one belonging from the G4 (91-120 days old) and two others belonging to the G5 (121-150 days old). Only two of the ninety one samples (2.20%), those belonging to the G2 (31-60 days old), were positive for *Neoscaris* sp. None of the samples was positive to rotavirus in PAGE technique.

Conclusions: The more frequent enteropathogens observed in buffalo calves were *Eimeria* sp., Strongylida, *Moniezia* sp., and *Neoscaris* sp., with *Eimeria* sp. being the most occurrent of the studied enteropathogens in the calf phase.

Parasitology

P04-004-072

Lasalocid supplementation and its effect on the control of *Eimeria* spp. parasitism in naturally infected Nellore calves in a tropical area of Brasil

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Objectives: The aim of this study was to assess the efficacy of lasalocid (1mg.kg⁻¹) administered over four months along with the protein and energy supplementation of calves, in a creep-feeding system, in the control of asymptomatic *Eimeria* spp. parasitism. This work also evaluated animal weight gain under the different treatment schemes.

Materials and Methods: Two hundred and eighty Nellore calves of both sexes, aging from four to six months, were randomly divided in two groups. Group one (T01) was comprised of 138 calves, and had a mean *Eimeria* oocyst count per gram of feces (OoPG) of 690.9 per animal. Group two (T02) was comprised of 142 animals, with a mean oocyst count of 692.2 OoPG. Both groups were supplemented in a creep-feeding scheme with a protein and energy concentrate. While animals in T01 had no further supplementation, those in T02 received 1.3 grams of lasalocid, mixed in the concentrate feed, for every 200 Kg body weight. Treatment begun two months prior to weaning, and continued for two months after weaning, during this period animals were assessed monthly through fecal oocyst counts (McMaster method), and weighed, to assess weight gain. To minimize competing effects from gastrointestinal nematodes, all calves were treated with moxidectin (200µg/kg) every 45 days. Mixed model procedure was used for the statistical analysis

Results: Regardless of the age of the animals, the calves of T02 (treated with lasalocid 1mg.kg⁻¹), showed a significant (P≤0.05) reduction of oocyst shedding per gram of feces in comparison to the control group, as early as one month after treatment started. This reduction, compared to the control, continued in all dates post-treatment. The efficacy rate above 95% (arithmetic means) was observed up to the 60th day after weaning. Furthermore, animals belonging to the T02 had an increase in weight gain throughout the period of 7.2 kg (p≤0.05), in comparison with the control animals (T01).

Conclusions: In conclusion, lasalocid (1mg.Kg-1) administered during one month was enough to reduce significantly (p≤0.05) the number of oocysts dispersing to environment. The four months addition of lasalocid was able to maintain the reduction of oocyst shedding per gram of feces. The control of these protozoan infections provides a significant increase in weight gain in comparison to untreated animals. This protocol could be recommended as a management tool to reduce the environmental dispersion of oocysts of *Eimeria* spp. minimising risks of reinfection, promoting an increase of health pattern.

Parasitology

P04-004-073

Fipronil and Fluzuron: efficacy of a novel combination against *Dermatobia hominis* and *Cochliomyia hominivorax* in naturally infested cattle

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Objectives: The efficacy of a new pour-on formulation comprising the anti-Parasiticides Fipronil (1.25 mg/ kg) + Fluzuron (2.5 mg/ kg) was evaluated in two field studies against *Dermatobia hominis* (Experiment 1) and *Cochliomyia hominivorax* (Experiment 2) in naturally infested cattle.

Materials and Methods: Experiment 1 was held on a farm in the municipality of São João da Boa Vista, São Paulo State, Brasil. In this study, 20 Canchim animals, naturally infested with *D. hominis* larvae (grubs) were randomly allocated into two groups of 10 animals each, based on nodule counts containing live larvae in the body of each animal on Day -1 pre-treatment: T01: Fipronil (1.25 mg/ kg) + Fluzuron (2.5 mg/ kg); T02: Saline 0.9% Control. After treatment, counts were carried on days 3, 7, 14, 21, 28, 35, 42 and 49 post-treatment.

Experiment 2 involved 12 calves divided into 2 groups of 6 animals. T01 was treated with Fipronil + Fluzuron and T02 was the control. Animals were randomly assigned to treatment groups, based on the individual weight measured at D-7. On day 0 (before treatment), each animal was subjected to two circular 4 cm skin incisions (one on each side of the body), between the infra-spinal cavity and the dorsal scapula border. This was done after sedation (dihydrothiazine hydrochloride) and local anesthesia (lidocaine 2%). Right after the surgery, treatment from T01 was carried out. Immediately after treatments, the 2 groups were kept in separate adjacent pastures, allowing natural exposure to *C. hominivorax* infestations. To evaluate the preventive efficacy of the new formulation, the 24 incisions were examined daily from the 1st - 10th DPT. The larvae in the lesions were classified as active (at least one live *C. hominivorax* larvae / lesion), and identified by a specific record with digit 1 (active) or digit 0, (not active, no live larvae in the lesion).

Results: The combination of Fipronil and Fluzuron in experiment 1 demonstrated efficacy higher than 90% from the 7th to the 35th day post-treatment (DPT), showing 99.5% of efficacy on 21 and 28 DPT. In all post-treatment dates, the *D. hominis* larvae counts were statistically lower (p ≤ 0.05) in the animals from T01 when compared to the control animals (T02).

In experiment 2, from the 1st -10th DPT, the Fipronil + Fluzuron reached 100% of preventive effectiveness, preventing T01 animals to develop active myiasis in all lesions. The animals in the control group showed

active myiasis from the 2nd to the 5th DPT, after which they had to be treated for animal welfare reasons (using a commercially available topical organophosphate).

Conclusions: With the results of these two studies it is possible to infer that the new formulation containing Fipronil (1.25 mg / kg) + Fluzuron (2.5mg / kg), in the dose and route employed, proved high efficacy against *Dermatobia hominis* larvae and preventive efficacy against *Cochliomyia hominivorax* in naturally infested cattle.

Parasitology

P04-004-074

Evaluation of the persistence of *Trypanosoma vivax* DNA after treatment with isometamidium hydrochloride

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Objectives: *Trypanosoma vivax* affects ruminants and equines in Africa, Central and South Americas, causing vast economic losses in affected herds. Clinical signs are unspecific and there are often aparasitemic periods that complicate diagnosis and treatment of affected animals. Treatment of ruminants infected with *T. vivax* is commonly performed with diminazene diaceturate (DM) and isometamidium hydrochloride (ISM), although resistance to both chemotherapy has been reported. This study aimed to evaluate the persistence of parasite DNA after treatment with ISM.

Materials and Methods: Three male goat (C1 to C3) experimentally infected with 2x10⁶ trypomastigotes of *T. vivax* were used. Blood collection was performed daily in D-2 and D-1 (pre-infection; PI), D1 to D14 (infected – Pretreatment; PRE) and D15 (treatment day) to D26 (infected – Post-treatment; POS). DNA extraction was performed using QIAamp DNAeasy Kit (Qiagen®), according to manufacturer's instructions and DNA stored at -20° C until use. Samples were subjected to cPCR that targets the gene cathepsin L-like enzyme (CatL) of *T. vivax* (Cortez et al., 2009) and to cPCR for the endogenous gene GAPDH (Birkenheuer et al., 2003).

Results: All samples were positive for cPCR to endogenous gene GAPDH, demonstrating the absence of reaction inhibitors and good quality of extracted DNA. On the other hand all PI samples were negative for cPCR to *T. vivax*. All PRE samples were positive for cPCR to *T. vivax*, confirming the infection. Parasite DNA was detected up to D24 (POS; nine days), but in alternate days and samples, being positive the C3 D16, C2 D17, C1 D18, C2 D18, C1 D21 and C2 D24 samples, other samples were negative for this test.

Conclusions: Our results indicate that the chemotherapy action on *T. vivax* is not immediate or that this hemoparasite may be resistant to this drug, which could favor the infection of others animals during this period.

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Parasitology

P04-004-075

Prevalence of *Tritrichomonas foetus* in bulls in The State of Chihuahua Mexico Tested with VetMAX™-Gold Trich Detection Kit

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Objectives: The aim of this study was to perform a pilot study in collaboration with the Facultad de Zootecnia from the Universidad Autonoma de Chihuahua Mexico with the implementation of the MagMAX™ sample preparation system and the USDA licensed VetMAX™-Gold Trich Detection Kit to investigate the prevalence and risk factors of *T. foetus* in the State of Chihuahua.

Materials and Methods: Four hundred and fifty breeding bulls from the state of Chihuahua Mexico were sampled with the TrichIT pipette by gently rasping the bull's prepuce. Inoculation of InPouch™ Biomed with smegma samples was carried over with an incubation time of 24 hours at 37°C. Nucleic acid extraction and purification was performed using the 5X pathogen DNA/RNA extraction and purification kit from Thermo Fisher Scientific as outlined by the kit manufacturer, utilizing a magnetic particle processor. Three hundred µl of sample was used in the extraction process. Their PEC, which also served as an IPC, was implemented by spiking 20,000 copies per extraction with Xeno DNA[®] into the lysis/binding solution used with each sample. Duplicates of a mock-purified sample referred to as the Xeno extraction control (XEC) were included in each plate processed. The XEC consisted of 300 µl of PBS processed in the same manner as the test samples. Final diagnostic calls were performed with the USDA licensed real-time PCR VetMAX-Gold Trich Detection Kit from Thermo Fisher Scientific.

Results: Results showed that from the 450 beef bulls tested, 113 were positive, which shows a prevalence of 25%. The high prevalence of *T. foetus* in the state of Chihuahua is similar to that reported in the mountains of Asturias in Spain (32%) in which cattle are managed in extensive pastoral systems where less than 2% of the matings are with artificial insemination (Mendoza-Ibarra et al., 2012). Differences of prevalence of *T. foetus* between the tested beef cattle breeds in this study were observed and may indicate possible resistant differences to the protozoa. In this sense several studies have shown that *Bos taurus* (beef) breeds have an increased risk to get infected when exposed to *T. foetus*, since they are up to six times more likely to get infected than *Bos indicus* breeds. The observed differences may be explained by the greater number of mounts performed by *Bos taurus* compared to *Bos indicus* in a similar time period, thus increasing the exposure of bulls to infected cows (Rae et al. 2004; Bondurant et al., 1990; Ortega-Mora et al., 1998). In addition we analyze age as a risk factor for the disease through the X² test, with an age range of 2-10 years. The data show that the differences observed in the presence of *T. foetus* and age was statistically significant (X² test, p < 0.001), in which bulls aged 10 years showed the highest percentage of parasite infection (78%).

Conclusions: The workflow of sample preparation with 5X MagMAX™ Pathogen RNA / DNA kit and real-time PCR-Gold VetMAX™ Trich Detection Kit, enabled the rapid and sensitive diagnosis of *T. foetus*. Its application allowed us to identify an important prevalence of the parasite (25%) in a population of beef bulls in the State of Chihuahua. Our data is consistent with others observed in similar beef production systems, where abortion control diseases through molecular diagnosis

is very limited or null. The urge to expand the diagnosis to control of *T. foetus* in the State of Chihuahua Mexico is demonstrated.

Parasitology

P04-004-076

Assessment of *Theileria* infections in ticks collected from the field by staining with methyl green Pyronin and Azure methods

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Objectives: Ovine theileriosis is an important hemoprotozoal disease of sheep and goats in tropical and subtropical regions that leads to economic losses in these animals. A rapid method is described for preparing and staining salivary glands of ticks infected with *Theileria lestoquardi*. This study was carried out to comparing between two staining methods for detecting of theileriosis in salivary glands of ticks.

Materials and Methods: A total of 200 suspected cases of ovine theileriosis were clinically examined in Tabriz in northwest of Iran. The sheep were investigated for the presence of *Theileria lestoquardi* in appropriate blood smears and any ticks species on body of sheep. Blood smears were stained by Giemsa method. Totally 240 adult ticks were collected from sheep. In laboratory *Hyalomma anatolicum*, *H. marginatum* and *Rhipicephalus sanguineus* were diagnosed. These ticks were examined for infection with *Theileria* by two staining methods. Whole salivary glands were stained with methyl green pyronin and Azure methods.

Results: In this study, 42(21%) of sheep were infected to *T. lestoquardi*. Infection in male sheep was higher than female. The examination of tick salivary glands showed that (7.5%) of salivary glands of ticks were positive to *Theileria* sporoblasts in methyl green pyronin staining method and this rate in Azure staining method was (6.66%). The prevalence rate of *Theileria* infection was higher in female ticks than in males but the difference was not significant ($P < 0.05$) probably because of smaller sample size.

Conclusions: The results of this study observed that is not significant differences between two methods of staining of salivary glands in ticks for detecting of infection to theileriosis. Base on the results of this study other staining method for diagnosing of theileriosis in ticks such as molecular procedures are recommended.

Comments: Base on the results of this study other staining method for diagnosing of theileriosis in ticks such as molecular procedures are recommended.

Parasitology

P04-004-077

Longitudinal survey of gastrointestinal parasitism in native cattle in the Central Dry Zone of Myanmar

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Objectives: Myanmar is undergoing rapid social and economic change. It is very reliant on agriculture, and agricultural development offers pathways for rural poverty alleviation. Many households own cattle, typically for draught, but which could provide meat and/or milk to regional markets. Little is known of cattle endemic disease, particularly those with subclinical effects, such as gastrointestinal parasitism. This study observed seasonal patterns of gastrointestinal parasitism in cows and calves in two villages in the Central Dry Zone (CDZ), and engaged local animal health workers in an example of a routine health and production monitoring program.

Materials and Methods: Longitudinal faecal sampling of cows and calves was performed in two villages—Kyauk Aoe ('KT', 20.80°N 95.59°E) and Ya Thar ('YT', 21.63°N 95.48°E)—representative of typical CDZ cattle production systems. The CDZ has a semi-arid tropical climate, with a long-term annual average rainfall of approximately 1000 mm that falls mainly between June and October, inclusive. November to January are cooler months of the year, with average daily temperatures rising to above 40°C in April prior to the onset of the monsoon in June.

Faecal samples were collected in September and December 2014, and January, March, July and October 2015, each time from approximately 30 cows (≥ 2 years old) and 20 calves (< 1 year old). These animals were randomly selected on each sampling occasion from 200–300 animals in each village enrolled in a larger study recording bi-monthly bodyweight and reproduction. Research project team members and community animal health workers recorded observations of animal health from ten broad syndromic categories, such as diarrhoea, lameness and mortality, each month.

Faecal samples were mixed with 3% formalin, equal to approximately 20% of the faecal sample volume, before transport to a government laboratory. Quantitative faecal flotation using the McMaster method was used to identify strongyle, ascarid and cestode eggs plus oocysts, and faecal sedimentation was performed to identify trematode eggs. Strongyle worm egg counts (WEC) were log-transformed and compared between months within villages using Student's t-test.

Results: Strongyle eggs and oocysts occurred at all sampling times. Trematode eggs occurred at all times except January (YT) and March (KA and YT). However, they were generally in $\leq 10\%$ of cow samples, except in YT in October (end of the rainy season) when 30% of cows were positive. Cestodes eggs occurred infrequently in low numbers.

Strongyle WECs were the most clinically important findings. Average cow WEC varied throughout the year but was similar in both villages within sampling months ($P \geq 0.09$). WEC was least in both villages in January in the early dry season (66 ± 25 (SEM) and 40 ± 14 eggs per gram (epg) in KA and YT, respectively). In KA, WEC in other months was significantly greater than in January (range 75–135 epg; $P \leq 0.045$). By contrast, in YT only March differed significantly from January (63 ± 12 epg; $P = 0.02$); WECs in other months were similar (range 41–87 epg; $P = 0.07$ – 0.99). Overall, 27–73% of cows had positive WECs at any sampling time. The top 25% of individual WECs were ≥ 100 epg at all times, except September–January in YT (75th percentiles of 88, 50 and 50 epg, respectively). Two preliminary larval cultures suggested the main species present were *Cooperia* and *Trichostrongylus*.

Average calf WEC and seasonal pattern were similar to cows, with average WEC lowest in January. However, fewer calves were sampled and there were fewer statistically significant differences between months, compared to cows.

Syndromic health records were generally inadequate although, anecdotally, signs that could be associated with gastrointestinal parasitism, such as diarrhoea, occurred infrequently.

Conclusions: This preliminary study's most striking finding was the relatively high strongyle WECs, despite the few signs of gastrointestinal parasitism. In cattle generally, nematode burdens with WECs less than those seen here may reduce growth rates or body condition scores. Thus, parasitism may be contributing to poor calf growth rates and/or cow condition scores, widely observed problems in the CDZ. Managing parasitism may help improve growth or cow reproductive efficiency, through improved condition score, and hence farmer incomes. However, more research is needed to identify efficient parasite management strategies and the size of treatment effects.

Parasitology

P04-004-078

Comparison between cPCR and qPCR for *Trypanosoma vivax* detection in cattle

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Objectives: *Trypanosoma vivax* is a hemoprotozoan that infects a wide range of ungulates, both wild and domestic animals and causes significant economic losses in cattle herds in tropical regions of Africa and Central and South Americas. The disease diagnosis is complicated by nonspecific symptoms such as anemia, abortion, diarrhea and weight loss. In this paper we compare the cPCR and qPCR for *T. vivax* DNA detection in three experimentally infected cattle (E1 to E3) with 2.0×10^7 trypomastigotes of *T. vivax*.

Materials and Methods: Three milliliters of whole blood was collected in 10% EDTA solution on -7, 0, 1, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 105, 112 and 120 days after infection (DAI). The DNA extraction was performed with the QIAamp DNAeasy Kit (Qiagen®), according to the manufacturer's recommendations. The extracted DNA was stored at -20°C until the moment of the use. cPCR was performed using a set of primers from the DNA sequence of the gene encoding for the cathepsin L-like enzyme (CatL) of *T. vivax* that had been described previously by Cortez et al. (2009). qPCR was performed using a set of primers from the DNA sequence of the gene encoding for the internal transcribed spacer 1 (ITS1) of *Trypanosoma* sp. and a specific probe for *T. vivax* that had been described previously by Silbermayr et al. (2013).

Results: Both techniques were able to detect the parasite DNA on 1 DAI. cPCR was able to detect 58.93% (33/56) of positive samples, detecting *T. vivax* DNA at 1, 7, 14, 21, 28, 35, 42, 49, 56, 63, 77 and 84 DAI for E1 bovine; at 1, 7, 14, 21, 28, 35, 42, 49, 56 and 63 DAI for E2 bovine; and at 1, 7, 14, 21, 28, 35, 42, 49, 56, 70 and 91 DAI for E3 bovine. Already the qPCR was able to detect 57.14% (32/56) of positive samples, detecting the parasite's DNA at 1, 7, 14, 21, 28, 35, 42, 49, 63, 77 and 84 DAI for E1 bovine; at 1, 7, 14, 21, 28, 35, 42, 49,

56 and 63 DAI for E2 bovine; and at 1, 7, 14, 21, 28, 35, 42, 56, 63, 70 and 91 DAI for E3 bovine. Furthermore Kappa coefficient was calculated between the two techniques, obtaining a value of 0.90, indicating an almost perfect agreement.

Conclusions: The use of qPCR for *T. vivax* detection is a recent technique and the results showed to be a useful tool for early diagnosis of trypanosomiasis. It is noteworthy the advantage of perform the absolute quantitation of parasitemia, obtaining the number of copies of the target gene.

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Parasitology

P04-004-079

Comparison Of Syringeability Of 3 Different Injectable Endectocides (Eprinomectin 20Mg/ML, Doramectin 10Mg/ML, Ivermectin 10Mg/ML)

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Objectives: Compliance in the use of an injectable product relies on different factors being one of them the syringeability. High fluidity allows fast injection which mitigates the risk of insufficient volume administration, needle breakage, spillage of product or injury of the user.

The aim of this study is to compare the ease of injection of 3 commercial endectocide injectables from the point of view of assessing their syringeability.

Materials and Methods: Three commercial endectocide injectables have been used in this trial: "Ivomec®" (Merial, Ivermectine 10 mg/ml), "Dectomax®" (Zoetis, Doramectine 10 mg/ml) and "Eprecis®" (Ceva, Eprinomectine 20 mg/ml). A volume of 15 ml of the tested product is withdrawn in a 20 ml glass syringe set up with a 1,2 mm or 0,8 mm diameter steel needles. A mass of 1 kg (equivalent to 1 Newton force) is applied on the piston and the time needed to empty the last 10 ml of solution is recorded.

This method is repeated 6 times for each solution at ambient temperature (20°C). Results provided are the average +/- standard deviation and statistical analysis.

Results: For the 1,2 needle, the times needed to empty the syringe were: "Ivomec®" (Merial, Ivermectine 10 mg/ml): 16,83 seconds +/- 0,75, "Dectomax®" (Zoetis, Doramectine 10 mg/ml): 22,83 seconds +/- 0,75, "Eprecis®" (Ceva, Eprinomectine 20 mg/ml): 12,2 seconds +/- 0,9. All these differences are significant (p > 0,05).

For the 0,8 needle, the times needed to empty the syringe were: "Ivomec®" (Merial, Ivermectine 10 mg/ml): 81,17 seconds +/- 3,76, "Dectomax®" (Zoetis, Doramectine 10 mg/ml): 115,83 seconds +/- 1,72, "Eprecis®" (Ceva, Eprinomectine 20 mg/ml): 52,7 seconds +/- 0,75. All these differences are significant (p > 0,05).

Conclusions: Good syringeability is important to facilitate the use of injectable products in the field and to ensure compliance. "Eprecis®" (Ceva, Eprinomectine 20 mg/ml) was demonstrated to have a significant better syringeability than the other two products tested with the two different needles tested. The superior performance can be explained by the active ingredient and the different excipients used in their formulation. This high fluidity strongly increases the comfort and convenience of use in the field.

Parasitology

P04-004-080

Use of thermographic images to detect external parasite load in cattlePaulo R. Dall' Cortivo¹Eduardo A. Dias¹Julio Otávio J. Barcellos¹Vanessa Peripolli²Concepta McManus²¹Animal Science, Federal University of Rio Grande do Sul, Porto Alegre, ²University of Brasília, Brasília, Brazil

Objectives: The aim of this study was to compare standard visual methods with infrared thermography combined with image analysis for automatic counting of ectoparasites (ticks and horn flies) in cattle under pasture conditions in Rio Grande do Sul state, southern Brazil.

Materials and Methods: Twenty Holstein cows, 10 Devon cows and 10 Braford bulls were used. Ectoparasites (ticks: *Boophilus microplus* and horn flies: *Haematobia irritans*) were counted visually and the animals were then photographed using an infrared camera (FLIR@ T300). This camera took simultaneous digital and infrared photographs so the accuracy of visual and thermographic methods were compared. A 1 cm metal object was placed on the side of the animal as a size reference. Photographs were taken from each animal at regions where the greatest number of ectoparasites were observed such as scrotum, flank, shoulder, face and perineum regions. The method used for counting ticks was based on the count of engorged females sized between 4.5 and 8.0 mm long by palpation on one side of the animal and the value was extrapolated to the other side of multiplying by two. For counting horn flies the methodology employed was visual counting one side of the animal and multiplied by two to obtain the total value. Three thermographic images and visual counts were obtained per animal. Images were first analyzed using standard Quick report® tools. This program also was used to alter the color palette from "rainbow" (camera default) to "inverted grey" to improve the image definition for counting parasites and then the program ImageJ® was used to automatically count the external parasites. Correlations between thermographic images and visual counts were calculated.

Results: Ectoparasites were cooler than the body of the animals in the thermographic images and therefore were easily highlighted by Quick report® program. In the case of ticks, they were especially visible when gravid female ticks were engorged. When small, they were frequently hidden under the animal hair, but in other regions, such as the face, they were easily seen. Horn flies stood out perfectly on thermographic images and were seen down to 3 mm in length. The correlation between visual and thermographic counts was 0.82 and increased to 0.90 when ImageJ® was adjusted to remove small dirt particles. Repeatability of measurements on the same animal was 0.87. No significant differences were seen between breeds of cattle for the ability to count horn flies using this method.

Conclusions: The use of thermographic images coupled with a software that contrasted points in the images was a useful tool and more accurate than the standard visual methods for counting external ectoparasites in cattle.

Parasitology

P04-004-081

Strategic Rhipicephalus microplus control through fluzuron associations reduces environmental and animal infestationIuri Vladimir Pioly Marmitt¹Samuel R. Félix¹Fabiane Niedermeyer¹Artur G. Nunes¹Bruno C. Chagas¹Elio Moro²Rogério Gama²Fernando Braga²Sergio S. D. Silva¹¹LADOPAR, Federal University of Pelotas, Pelotas, ²Zoetis Brasil, São Paulo, Brazil

Objectives: The aim of this study was to assess the parasite control program proposed by Zoetis™, comprised of an association of benzoylphenyl ureas (fluzuron) and systemic macrocyclic lactones (ivermectin doramectin) or (milbemycin moxidectin), against the tick *Rhipicephalus microplus*, compared to the lone, traditional tactical interventions of topical organophosphates and synthetic pyrethroids (Cipermetrin and Clorpirifos). Furthermore, we assess the reduction in environmental infestation; cattle infestation; and the number of tactical interventions throughout the year.

Materials and Methods: The present study was conducted in Piratini (31 °S), Rio Grande do Sul state, Brazil; from November 2013 to October 2014. Cattle included were 13 month old females, weighing 265Kg (±19Kg), all of Angus breed. These heifers were maintained under the same conditions, in a region of high environmental concentrations of *R. microplus*. Seventy eight heifers were randomized according to tick counts and live weight, and assigned to one of three treatment groups (N=26 per group). These groups were further split in two (n=13), and assigned to six independent pasture areas, to account for field variation. The groups were treated with either a combination of topical Fluzuron and subcutaneous Moxidectin (FLU+MOX); or Topical Fluzuron and subcutaneous Doramectin (FLU+DOR); and an untreated control group. Groups FLU+MOX and FLU+DOR were treated according to the manufacturer's instructions. Every 14 days the cattle were submitted to a tick count of the entire left side of the body. Whenever the mean count for a group was over 30 ticks/heifer, that group was pulverized with Cipermetrin and Clorpirifos (as instructed), called as tactical intervention. The environmental infestation was assessed through the total number of ticks counted in each group after the Fluzuron residual effect had expired (day 185). Likewise, individual animal infestation was considered every 14 days, and a mean was generated for each group. The third parameter considered was the number of tactical interventions necessary, for each group, throughout the year. Data were compared using Statistix 9.0 or EpiInfo 7.

Results: Environmental infestation was regarded as the tick count after the residual Fluzuron effect (day 185). Mean Tick counts per animal for the period from day 185 to day 364 were, for group FLU+MOX = 137.6 (±42.1); FLU+DOR = 196.2 (±61.6); and Control = 218.7 (±92.6). The pastures where the animals treated with FLU+MOX were kept were statistically less infested, when compared to the other groups (p<0.01), the other groups did not differ among themselves (Kruskall-Wallis test). Two different parameters were considered to assess animal infestation: Mean tick count per animal throughout the year; and best performance in each individual assessment. Group FLU+MOX had the lowest overall mean tick count, at an average of 8.7 ticks/animal/assessment (p<0.01). Group FLU+DOR had a count of 13.6, which was a performance better than the control, at 40.6 (p<0.01) (Tukey test). Furthermore, animals in group FLU+MOX had a statistically lower (p<0.01) tick count on 16 (of 28) assessments, compared to the control, and 10 times was it better than the FLU+DOR group. Conversely, FLU+DOR had lower counts (p<0.01) on 17 and 5 occasions when compared to the Control and FLU+MOX, respectively (Tukey test). The number of tactical interventions necessary

was greatly reduced in the Fluzuron treated groups, using FLU+MOX requiring two interventions and FLU+DOR requiring eight, while the control had 16. In this analysis FLU+MOX performed better than the control, representing a protection factor (OR=0.093, 0.02-0.43) ($p<0.01$).

Conclusions: Topical Fluzuron based treatments promote a sharp reduction in the environmental infestation of *R. microplus*; nonetheless, in the period assessed, they are not able to fully eliminate the tick from the environment. The association of Fluzuron and Moxidectin is the most efficient for environmental tick control. This association is also more efficient in reducing tick populations on the animal. Both Fluzuron based treatments outperform the control group in all parameters. Furthermore, the association of topical Fluzuron and Moxidectin reduces the need for tactical interventions, when compared to the control (OR 0.093).

Parasitology

P04-004-082

Prevalence of paramphistomosis in cattle slaughtered at São Miguel Island abattoir, Azores, Portugal

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Objectives: The aim of this work was to calculate the rumen fluke prevalence at slaughtered cattle, the geographical distribution of these parasites by herd and county in order to identify the most affected areas of SMI and investigate if *Paramphistomum* sp. and *Fasciola hepatica* share the same intermediate host, *Lymnaea truncatula*.

Materials and Methods: São Miguel Island (SMI) is the largest of the nine islands that composed the Azores archipelago, which is located in the North Atlantic Ocean (36 to 39°N, 25 to 31°W). The climate is oceanic, with 900 to 3000 mm of rain annually, and mild (17°C, range 9 to 26°C), with small temperature differences between summer and winter, and no frosts below 600 m altitude. There are about 108,000 cattle on SMI of which 52,000 are dairy cows, almost all being of Holstein-Friesians. There are 1400 dairy herds with an average of 37 cows per farm. It was possible, during meat inspection at the sole SMIA, to identify the presence of *Paramphistomum* adult forms in the rumen mucosa of 608 cattle of a total of 54144 bovines slaughtered from April 2014 to September 2015 (18 months). The geographical distribution was based on the bovine identification ear tag and two databases sources: National System of Identification and Animal Record (SNIRA) and the Information System of Agricultural Farms of the Azores (SIARA). The output cartograms were developed using Geographic Information System (GIS) and ArcGIS Desktop-ArcMAP softwares.

Results: Rumen fluke was unknown in cattle reared at the Azores archipelago until April 2014. At this time adult forms of parasite were recognized in the ruminal mucosa for the first time at SMI abattoir. Of the 608 diagnosed cases, 542 were seen in females (89.1%) and 66 males (10.85%). The prevalence of rumen flukes on cattle slaughtered population on SMIA was 1.12% being 2.77% in females and 0.19% in males. Most of rumen flukes cases were observed in females from three to nine years old and from six months to two years old in males. It was also noted that prevalence has seasonal variations, reaching high levels on summer months. The counties with the highest prevalence rates were those located in the eastern half of SMI and more than 30% of dairy herds located in these areas are affected by rumen fluke.

The geographical distribution of rumen flukes and liver flukes cases indicates that these parasites can share the same intermediate host, *Lymnaea truncatula*, at the eastern side of the Island. However, there are more cases of rumen fluke than liver fluke on the western half of SMI, where *Lymnaea truncatula* was never identified. This fact implies that exist another intermediate hosts, not yet identified, for rumen fluke. Females are more affected than males because they live more years and spend more time grazing. Most of males are reared in feedlots and are slaughtered before two years old.

Conclusions: In spite of low prevalence of rumen fluke on SMI cattle population, farmers and veterinarians should be aware of this disease, especially in areas where fasciolosis is more prevalent. The weather conditions, grazing on waterlogged pastures and areas prone to flooding, and lack of deworming practices appropriate for rumen flukes are significant risks factors for the development and spread of rumen flukes in SMI cattle.

Parasitology

P04-004-083

First Report of Paramphistomum Leydeni in Irish Sheep Flocks

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Objectives: Paramphistomosis is an important parasitic disease of ruminants caused by different species of Paramphistomidae family, which can result in decreased production and even death. Previous studies suggested that *Calicophoron daubneyi* is the predominant species affecting cattle in Europe, including Ireland. Data are limited, however. Increasing our knowledge of trematode species is essential for epidemiological surveys, as different species may have different host ranges, variable pathogenicity and anthelmintic resistance. The aim of this study was to characterise the species of rumen fluke in Irish sheep flocks using isolated eggs.

Materials and Methods: A subset of 14 Irish sheep flocks recruited to a rumen fluke prevalence study was identified for further investigation. These commercial farms recorded the highest faecal egg counts of over 250 flocks examined. Each of the 14 farmers submitted 20 catch samples from mature ewes in February 2015. These samples were subsequently pooled into four composite samples, each composite representing five individual samples. Samples were concentrated by sedimentation with eggs subsequently washed in distilled water. Rumen fluke eggs were individually collected under stereomicroscope using a modified Pasteur pipette. Samples were subjected to 3 freeze-thaw cycles at -80°C for 30 minutes followed by boiling at 100°C for 10 minutes. DNA was extracted from eggs using the QIAamp® DNA stool mini kit (Qiagen, Hilden, Germany) and quantified spectrophotometrically at 260nm. DNA samples were stored at -80°C until further analysis.

Species identification was completed by molecular analysis of 54 individually frozen eggs randomly selected from 14 different farms. The ITS-2 nuclear region was amplified by PCR using the following primers; forward 5-TGCATACTGCTTTGAACATCG-3 and reverse: 5-GTTCAGCGGGTATTACGTC-3. Amplified products were separated electrophoretically in an agarose gel and purified using a Qiaquick Gel extraction kit (Qiagen, Hilden, Germany). Purified products were forward and reverse sequenced by the Sanger method

using the same PCR primers. Sequences were compared to those available in GenbankTM, using the BLASTn (NCBI) algorithm for specific identification.

Results: The average concentration of DNA isolated from an individual egg was 3.5ng/μl (range 1.1-12.3ng/μl). The PCR technique allowed the identification of eggs individually isolated from faecal samples. All amplified PCR products were approximately 400bp in size.

All samples sequenced contained the complete ITS-2 region plus flanking 5.8S rRNA and 28S rRNA partial sequences. Of the 54 samples analysed, 52 (96.29%) were identified as *C. daubneyi*. All had identical sequences and showed 100% homology with region ITS-2 of *C. daubneyi* (GenBank accession no. KP201674.1). The remaining two samples were identified as *Paramphistomum leydeyni*. The primers used produced a fragment of 402 pb which showed 100% homology to previously published sequences of the ITS-2 region of *P. leydeni* from northern Uruguay (GenBank accession no. KJ995529.1, gb|KJ995526.1, KJ995527.1) and Argentina (GenBank accession no. HM209064.1). All *P. leydeni* sequences were identical and displayed no intraspecific variation.

Conclusions: This study constitutes the first report of *P. leydeni* infecting sheep in Ireland. The implication of this unexpected outcome remains unclear, however. Further studies are required to elucidate if this is an incidental finding of no clinical significance, or whether both *P. leydeni* and *C. daubneyi* are fluke species common in Irish flocks.

Parasitology

P04-004-084

Dynamic anaplasmosis in dairy calves in intensive farming system

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Objectives: The Complex Bovine Parasitic Sadness annually causes great economic losses, anaplasmosis is the main pathology responsible for these losses. The aim of this study was to evaluate the dynamics of anti-*Anaplasma marginale* antibodies in different age groups cattle.

Materials and Methods: This study was conducted in a specialized intensive dairy farm. The blood of 221 Holstein animals divided into seven groups was collected by puncturing the jugular vein, 42 younger calves in the suckler group, 31 older calves in the suckler group, 27 post-weaning, 31 calves risk group, 30 calves at the stage where they get sick, 30 animals in the stable group and 30 cows in the pre-partum period. Indirect fluorescent antibody test was performed using the serum obtained to detect anti-*Anaplasma marginale* antibodies.

Results: On evaluation in epifluorescein microscope, in the objective lens of 20X and 40X increase, in indirect fluorescent antibody test for *Anaplasma marginale* in dilution 1:40 were detected 72 % in younger calves in the suckler group, 58 % in older calves in suckler group, 59 % in the post-weaning group, 39 % in the risk animals group, 27 % in the group of sick animals, 83 % in the stable animals group and 86 % in the group of pre-partum cows.

Samples were considered negative when they have not fluorescence in dilution 1:40.

Conclusions: The study demonstrates the dynamics of anti-*Anaplasma*

marginale antibodies and contain the newborn animals still passive immunity via colostrum received and the age increase these antibodies are decreasing reaching the window period. The risk groups and sick, antibodies are very low, further increasing the stable animals group, thus indicating the formation of active immunity.

Parasitology

P04-004-085

Survey of endoparasites and their control on goat farms in Ireland

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Objectives: The Irish goat industry is quite small – only about 1,014 goat keepers with a total of 15,638 goats (December 2014 census). Parasite and parasite control therefore receive very little attention within this sector. As with all grazing ruminant, gastrointestinal nematodes (GIN) remain one of the main constraints to production. The purpose of this study was to examine worm control practices and use of antiparasitic drugs in goats. A second aim was also to determine the main nematode parasite groups infecting Irish goats.

Materials and Methods: A short questionnaire survey with standard questions relating to the characteristics of the farm, the management of goats, the worm control practices and the anthelmintics used were distributed to goat farmers. Farmers were requested to complete the survey and submit faecal samples from 15 adult (> 12 months) and 5 juvenile (4-12 months) goats for analysis. Faecal worm egg counts (FEC) were performed on pooled faecal samples (5 animals per group) using the FLOTAC technique having an analytic sensitivity of 6 eggs per gram of faeces (epg).

Results: Questionnaires were sent to 64 goat farmers and 11 (17%) returned completed questionnaires with faecal samples. The majority of respondents (n=6; 55%) indicated that the main farming enterprise was dairy/cheese production. Not surprisingly, on 45% of farms, goats were kept indoors all the time. Anthelmintics were used on 72% (n=8) of farms but on the majority of farms no predetermined drenching programme were followed. Only 50% of farms used average weight of the group to determine the anthelmintic dose, which in the majority of cases (n=6) was 1.25-2x the recommended sheep dose. Overall 91% (n=10) of the farms were positive for strongyle eggs with a range of 24-1050 epg. All farms sampled (n=11) were positive for coccidial oocysts (range = 12-6480 oocysts per gram). Only 1 farm was positive for *Fasciola hepatica* and 3 farms for rumen fluke -eggs (range 18-264 epg).

Conclusions: These results indicate that exposure to nematodes and coccidia is high in Irish goat farms despite the fact that 45% of the goats are kept indoors all the time. Furthermore there is a high risk of under-dosing of anthelmintic drugs due to inaccurate weight calculation to determine the amount of drug to use. Thus the danger for the development of anthelmintic resistance exists and needs further investigation and farmers need more information on appropriate parasite control practices.

Paratuberculosis

P04-004-086

Post-mortem evaluation of fourteen adult cattle with suspected Johne’s disease based on herd disease status and/or suggestive clinical signs

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Objectives: Johne’s disease is caused by infection with Mycobacterium avium subspecies paratuberculosis (MAP) and leads to a chronic wasting condition in adult cattle. The immune response to MAP infection is not fully understood, which limits the performance of currently available diagnostic tests. Only a small proportion of MAP-infected cattle are thought to become clinically affected and factors which may predispose to the advancement of clinical signs remain unknown. Post-mortem investigation of naturally infected animals informs both individual diagnosis and on-farm management policies.

Materials and Methods: Between 2013-2015, fourteen adult cattle (thirteen dairy animals [nine Holstein-Friesians, two Jerseys and two Holstein-Friesian/Jerseys] and one beef bull [Simmental]) with suspected MAP infection presented to University College Dublin (UCD) for post-mortem evaluation. The cattle came from six different herds, five of which had a history of Johne’s disease. Some of the animals had been screened for MAP using various tests; six had evidence of MAP antibodies in serum or milk samples, four were MAP faecal culture positive and three were MAP faecal PCR positive. Nine animals had developed clinical signs consistent with MAP infection. The cattle were euthanized and underwent immediate post-mortem examination. Tissue samples from the ileocaecal valve and ileocaecal lymph node of all animals were processed for histopathology and stained using both haematoxylin and eosin (H&E) and the Ziehl Neelsen (ZN) method. Faecal samples from all animals were cultured for MAP (TREK ESP® para-JEM® system).

Results: Seven cattle were diagnosed with clinical Johne’s disease based on the presence of characteristic gross pathological and histopathological lesions within the ileum and MAP faecal culture results; five of these also showed evidence of moderate to severe abomasal damage caused by the parasite *Ostertagia ostertagi*. The gross and histopathological lesions within the ileum of another animal were highly unusual; although the positive faecal culture result indicated that the animal was probably MAP infected, an underlying neoplastic or immune-mediated cause of the lesions could not be discounted. This animal also had evidence of severe Type II ostertagiasis within the abomasum. One animal was diagnosed with lymphocytic-plasmacytic enteritis. Two animals were diagnosed with traumatic reticulo-peritonitis (‘hardware disease’), one of which had evidence of mild Type II ostertagiasis within the abomasum. No diagnosis could be reached for three animals.

Conclusions: These case reports illustrate that the ante-mortem diagnosis of Johne’s disease in cattle remains challenging. Clinical signs caused by MAP are relatively non-specific and may be due to other disease states; it remains important to consider all differential diagnoses. MAP may exist on farms concurrently with other herd health problems that require management; further study is necessary to elucidate potential connections between MAP and other factors, such as parasitism. Ultimately, there is an urgent requirement for the development of improved diagnostic tests for the identification of MAP infection in cattle.

Paratuberculosis

P04-004-087

Comparison of Johne’s disease prevalence on organic and conventional dairy farms in Pennsylvania.

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Objectives: To determine is Johne’s disease (JD) prevalence on Pennsylvania (PA) organic dairy farms is different than that of conventional dairy farms. To identify differences in management practices between organic and conventional farms. To identify risk factors associated with a high prevalence of JD on organic farms, compared with organic farms that have low prevalence, and to make similar comparisons for conventional farms.

Materials and Methods: Fifty PA dairy farms (26 certified organic, 24 conventional) were enrolled. Individual milk samples were collected as part of the monthly somatic cell count testing. Milk was tested for Mycobacterium avium subsp. paratuberculosis (MAP) antibodies using a commercially available ELISA kit. Information regarding pertinent management practices was gathered during a farm visit conducted by a single trained observer. Basic statistics were used for farm demographics. Overall herd prevalence and within-herd prevalence were compared between groups. Multivariate analysis was employed to look for risk factors and associations.

Results: A total of 2,739 cows were included in the study. Organic herds had a median of 39 lactating cows (range, 20-211 cows); while conventional herds had a median of 58 lactating cows (range, 20-114 cows). Total herd size was significantly higher in the conventional group (median, 128 heads of cow) versus organic group (median, 78 heads) (p=0.018). Average daily milk production was significantly higher in the conventional group (mean, 71 lbs/day) versus the organic group (mean, 49 lbs/day)(p=0.051).

Between-herd prevalence was not statistically different between herd types with 13/26 (50%) positive organic herds versus 14/24 (58%) positive conventional herd. Among positive herds, the proportion of JD positive cows tended to be higher (yet, not statistically significant) in organic herds (2.3%) than in conventional herds (1.6%).

Risk factors associated with increased prevalence on organic farms included sharing of pasture and water source between replacement stock and adult cattle, the lack of use of bedding in replacement stock, and the lack of routine vaccination. Risk factors associated with increased prevalence on conventional farms included purchasing of animals, sharing of pasture or water source between replacement stock and adult cattle, feeding of whole milk, and the use of group maternity pens.

Conclusions: The prevalence of JD on PA organic farms is no different than that of conventional farms matched by size. The differences in management practices between organic and conventional dairy farms do not appear to influence JD prevalence within the herds.

Paratuberculosis

P04-004-088

Circulating MiRNAs In Bovine Serum and their Potential Use as Novel Biomarkers of Early Mycobacterium avium subsp paratuberculosis Infection

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Objectives: Johne's disease is a chronic enteritis of ruminants caused by *Mycobacterium avium* subspecies paratuberculosis (MAP). New disease control tools are needed as current strategies are hampered by the lack of sensitive and specific diagnostic modalities. Circulating microRNAs (miRNAs) have been shown to have potential as novel biomarkers for various human diseases, but their potential application in the veterinary sphere has not been explored. The aim of this study was to use RNA-sequencing to assess the potential of miRNAs as biomarkers for JD disease progression.

Materials and Methods: Sera from a six month experimental infection conducted at UCD consisting of MAP-challenged calves (n=6) and age-matched controls (n=6) was used. We also analysed sera that had been stored at -20°C for over a decade from a four year MAP infection model performed by the Central Veterinary Institute (CVI) of Wageningen University. Cattle defined as seropositive for anti-MAP antibodies (n=5) were compared against sero-negative cattle (n=7). Comparison of UCD and CVI samples also enabled us to assess the stability of miRNA profiles in biobanked sera. The animal work was approved by the Animal Research Ethics Committee of each university and experimental procedures were performed according to appropriate animal welfare legislation.

Results: From the UCD samples, we identified 100-200 known miRNAs with multiple isomiRs and 30 novel miRNAs. Surprisingly, the small RNA profile was highly similar to that of the biobanked CVI samples. No significant differential miRNA expression was detected between MAP-infected and their age-matched controls in either experiment. In contrast, comparing pre-infection sera of young calves to the 6 month interval of each experiment revealed miR-29a, miR-92b and miR-205 increases (2-fold) that may be due to blood-cell population changes during calf maturation (P<0.001).

Conclusions: Our study has demonstrated the stability of miRNA in serum, identified a range of novel miRNA in bovine serum, and shown the utility of small RNA sequencing approaches to explore the potential of miRNA as novel biomarkers for infectious disease in cattle.

Paratuberculosis

P04-004-089

Genetic Markers of Resistance to Johne's Disease: Their Validation and Functional Assessment

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Objectives: Johne's disease (JD) is a bacterial disease of ruminants, caused by *Mycobacterium avium* subsp. paratuberculosis. Resistance to JD has been shown to be heritable and many candidate gene and GWAS have identified QTLs and SNPs associated with resistance or susceptibility to JD in cattle. However, validation of these markers takes precedence prior to their inclusion in genetic selection indices to breed for JD resistance. Therefore, the objective of this study is to validate previously associated genetic markers in a new resource population, and functionally characterize their biological significance in vitro using a bovine macrophage cell line.

Materials and Methods: A total of 1000 cows from Johne's positive herds will be genotyped using the Geneseek GGD panel that contains 33,000 SNPs plus additional SNPs that were previously associated with JD. Further associations with milk somatic cell score (SCS), protein and yield, as well as fertility traits will also be assessed. To validate the biological significance of SNPs in vitro, a bovine macrophage (BOMAC) cell line will be genome edited using CRISPR/cas9 to incorporate individual SNPs in genes of interest (i.e. IL10RA), previously associated with JD resistance. Functional assessment of SNPs will be determined by characterizing their BOMAC phagocytic activity and cell signalling in response to *Mycobacterium avium* subsp. paratuberculosis and other immunostimulants (i.e. MDP or PMA + ionomycin). Total RNA will be extracted from stimulated and gene-edited BOMACs over time to profile gene expression by next-generation sequencing.

Results: The resource population for the genetic association study is currently being established based on serum ELISA, fecal PCR and/or fecal culture testing. Crispr/cas9 gene editing methodology has been used to insert a SNP in the IL10RA gene (633C>A) into the BOMAC cell line and the insertion has been validated by sequencing. Infection studies to test the biological significance of these SNPs are currently underway. Other genes of interest for genome editing of BOMACs by Crispr/cas9 include TLR4, IL23R and IL12R.

Conclusions: Johne's disease is implicated with severe economic losses to dairy industry and is also attracting significant public health concern. The disease is distributed worldwide and a possible way of its control is through breeding animals for enhanced resistance. Currently there is a knowledge gap concerned with the validation of previously associated genetic markers before they could be incorporated in genetic selection. Validation and assessment of biological significance of these markers will provide evidence that could aid in their inclusion in future dairy breeding programmes.

Paratuberculosis

P04-004-090

Prevalence of Mycobacterium avium subs. paratuberculosis in Portuguese dairy herds

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Objectives: To establish the proportion of dairy herds (members of the Portuguese Dairy Record Association ANABLE) infected by *Mycobacterium avium* subsp paratuberculosis (MAP) and its distribution at municipality level.

Materials and Methods: From the total of 1263 farms registered in ANABLE data base (ADB), 222 (DB) were classified according to MAP infection status. In these herds all cows aged over 30 months, were MAP tested by indirect biphasic ELISA. Individual results were negative (NEG), positive (POS) or dubious (DUB). Farm level of infection was defined based on all adult cows test as Negative (NEG) if all cows were negative, and Positive (POS) if otherwise. Farms from ADB were plotted in ArcGIS Vs10 at the municipality level, and proportion of infected herds was determined and represented at the same level. The sampling fraction was determined and used to correct the potential sampling bias.

Results: Our sample comprised 18% (222) of the ADB farms and 43.2% (48) municipalities (Mnc). The 48 municipalities comprise 1074 (85%) of the total 1263 farms in ADB. The global proportion of infection was 58% (CI 95%:±3). Prevalence at municipality level ranged from 0 to 100%. The proportion of positive farms was zero in 25% of the Mnc. Positive Mnc had a proportion of infected samples that ranged from 1% to <33% in 50% of the Mnc; 33% to <67% in 18.7% of the Mnc and >67% in the remaining 6,3%.

Maps representing the sampling fraction and proportion of infected farms were produced for the municipalities and districts levels. Inferences for prevalence distribution of MAP in dairy herds in Portugal are presented. Previous studies reported in 2004 49.5% of MAP herd prevalence in the north of Portugal. This is the first effort to assess the continental prevalence of MAP in the country.

Conclusions: This study supports a high global level of MAP infection in Portuguese dairy herds, but important heterogeneity at the municipality level. Compared to previous reported prevalence, this study suggests the possibility of a higher national prevalence.

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Paratuberculosis

P04-004-093

Evaluation of the diagnostic value of colostrum antibody ELISA for early detection of Mycobacterium avium subsp. paratuberculosis in dairy cattle

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Objectives: Early diagnosis of subclinical Mycobacterium avium subsp. paratuberculosis (MAP) infected animals is important to control and monitor paratuberculosis in dairy herds; however, there is a lack of reliable diagnostic tests for early detection of MAP.

The objectives of this study are to evaluate the performance of a commercial milk MAP antibody ELISA in first day colostrum and to compare the diagnostic value of milk MAP antibody ELISA in colostrum to that of standard serum antibody ELISA in early detection of MAP infection in cattle.

Materials and Methods: Colostrum samples were taken between 0 and 4 days-in-milk (DIM), and serum samples were collected during the spring calving period in 2015 from 377 mixed-parity cows in a MAP positive commercial Jersey dairy herd. Lactation and body weight records were obtained during the trial period to match them with results of individual animal diagnostic testing for MAP. Sample analysis is still ongoing at this point. The odds for colostrum samples testing positive in a MAP-specific Immunoglobulin G (IgG) ELISA as a function of covariates parity and

DIM will be evaluated using logistic regression. Sensitivity and specificity of the colostrum-based ELISA will be determined using serum ELISA results and information from individual cow performance as references.

Results: The apparent seroprevalence in the herd was 4.77%. The majority (78%) of seropositive animals were four years of age or older. Two seropositive animals and one animal with an equivocal test result were two years of age, indicating high levels of exposure within the herd. The average body condition score of seropositive cows was 4.44 out of 10 which was above the herd average of 4.39 out of 10. Sample and data analysis are not completed at this point; further results will be presented at the congress.

Conclusions: First day colostrum samples are easily obtained and may provide a promising alternative to serum antibody ELISA for early individual animal testing if the test sensitivity and specificity are acceptable.

Paratuberculosis

P04-004-094

Long term survival of animals positive to MAP diagnostic tests in Ireland

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Objectives: To determine longevity of animals positive on a seroprevalence survey for Mycobacterium avium paratuberculosis and of animals positive on a faecal test submitted on clinical animals to the Regional Veterinary Laboratories.

Materials and Methods:

Three groups of animals were included in the survival analysis.

1. Animals positive on the 2005 seroprevalence survey (Good et al., 2009)
2. Animals positive on a faecal test submitted on clinical animals to the Regional Veterinary Laboratories in 2005.
3. Two control animals were matched with each positive animal. These animals were picked from herds that had been tested negative on the prevalence survey and were matched on enterprise type, herd size and age of animal.

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Results: 201 animals were positive on the prevalence survey. 8 were still alive eight years later. Of the 192 animals that were dead, one had been exported, 13% had died on the farm and 85% were sent for slaughter. Of the 400 control animals twenty five animals were still alive 8 years later. Of the 375 animals that were dead, three had been exported, 13% had died on the farm and 85% were sent for slaughter.

Animals positive on a faecal test.20% had been sold. 20 were sent to the slaughterhouse, 18 died on the farm and one animal was exported. Animals survived from 1 to 1056 days after a positive faecal test.Of the 80 control animals 5.7% were still alive 8 years later. Of the 74 animals that were dead, one had been exported, 16% had died on the farm and 84% were sent for slaughter.

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Conclusions: The survival of positive animals on the prevalence survey compared with controls shows that on average positive animals died a year sooner than controls. Of the 40 animals positive on the faecal sample, the great majority were dead within 250 days of the sample being taken whereas 5.7% of the control animals were still alive five years later.

Paratuberculosis

P04-004-095

Applying Sociology to the control and prevention of Paratuberculosis

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Objectives: This paper presents the framework and indicative outcomes of an interdisciplinary project involving veterinarians and sociologists. Responding to the ongoing challenge of improving the uptake and effective implementation of herd health programmes at farm-level, the paper utilises social science to investigate farmer behavioural factors. The paper identifies key categories of sociological data that are directly relevant to informing a HACCP-based herd health programme for the control and prevention of bovine paratuberculosis.

Materials and Methods: We used in-depth qualitative methodologies to identify farmers' values and knowledges relevant to animal disease. The utility of such a methodological approach has been established in research that has analysed implementers' engagement with HACCP (Lowe and Taylor, 2013) and the social and cultural aspects of herd health management at farm level (Vanclay and Enticott, 2011). Qualitative methodologies, rather than eliciting interviewees' self reported data, involve the researcher uncovering values, beliefs and practices

and analysing them. In this study we used the Biographic Narrative Interpretive Method (BNIM) (Wengraf, 2001) to undertake twelve case studies that represent a wide range of farm holders (with and without a prior diagnosis of paratuberculosis). In addition, ethnographic research was undertaken, which involved observing farmers' implementation of herd health measures in practice on farms known to be currently infected with paratuberculosis. The data were transcribed and analysed using a framework of values and knowledges concepts

Results: Animal disease such as paratuberculosis, has cultural (pride), social (relationship) and economic (monetary) costs for farmers. On the other hand, optimal animal health gives rise to social, cultural and economic benefits. Thus, farmers, can be diversely motivated to implement herd health programmes. Effective promotion of these requires communicating not only the economic but also the cultural and social benefits of effective implementation (and costs of non-implementation). Furthermore, farmers' knowledge constructs (claims, lore/scripts and practices/practical consciousness) in relation to animal disease are identified. Farmers have professionally or scientifically derived information (claims) in relation to animal disease, which they believe is factual. In addition, lore and myths (scripts) can circulate within the farming community in relation to animal disease. Also, farmers have habits or pragmatic constraints (practical consciousness) that impact their management of herd health measures. We argue that in order to address barriers and exploit opportunities for successful implementation, the design of herd health measures and veterinary advice must engage with knowledge constructs (claims, scripts and practical consciousness) in a targeted way. Some knowledge constructs are obstructive to effective herd health management and thus must be challenged by policy makers and veterinary practitioners. Others are supportive to herd health and can be strategically promoted within the farming community.

Conclusions: This paper has introduced an extended suite of sociological concepts and methodologies that, to the authors' knowledge, have not been utilised in the literature on farm-level herd health heretofore. Informed by the sociology, herd health and food safety literature, our framework can be applied to inform the design of wide-ranging herd health and HACCP-based programmes. Areas for further work include broadening the research focus to include the knowledge constructs of other key stakeholders in animal health, including veterinary scientists/practitioners and policy-makers and examining the intersections between them.

Paratuberculosis

P04-004-096

Bayesian estimation of prevalence of paratuberculosis in dairy herds enrolled in a voluntary Johne's Disease Control Programme in Ireland

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Objectives: Bovine paratuberculosis is a disease characterised by chronic granulomatous enteritis which manifests clinically as a protein-losing enteropathy causing diarrhoea, hypoproteinaemia, emaciation and, eventually death. Some evidence exists to suggest a possible

zoonotic link. A national voluntary Johne's Disease Control Programme was initiated by Animal Health Ireland in 2013. The objective of this study was to use Bayesian methodology to estimate herd level prevalence of paratuberculosis in Irish herds enrolled in the national voluntary JD control programme during 2013-14.

Materials and Methods: Two datasets were analysed in this prevalence study. An initial analysis utilised data that had been collected in Ireland during 2005 (including 5822 test results from 119 herds), and was used to construct model priors. Model priors were updated in a Bayesian model with data from a primary (2013-14) dataset. The primary dataset was generated as part of the Animal Health Ireland voluntary Johne's Disease Control Programme and included test records from 99101 animals across 1039 Irish dairy herds.

Results: Overall, 57% of herds in the primary dataset had at least 1 positive test result. Preliminary analyses suggest a median posterior estimate (95% credible interval) for herd prevalence of approximately 0.28 (0.23, 0.34), 10th, 20th, 75th and 90th percentiles were 0.25, 0.26, 0.30 and 0.32 respectively. Across all herds the average within herd prevalence was found to be 0.043 (0.037, 0.050). A sensitivity analysis demonstrated that the final estimate was most sensitive to the prior for the prevalence within infected herds.

Conclusions: This study represents the first use of Bayesian methodology to estimate the prevalence of paratuberculosis in Irish dairy herds. Analyses are ongoing, however, preliminary results suggest that the herd level prevalence estimate for dairy herds enrolled in the voluntary national Johne's Disease Control Programme is higher than previous Irish national estimates, but still lower than estimates from other major dairy producing countries.

Paratuberculosis

P04-004-097

Control of *Mycobacterium avium* subspecies paratuberculosis (MAP) in an Irish context

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Objectives: Johne's disease (JD), caused by *Mycobacterium avium* subspecies paratuberculosis (MAP), continues to present diagnostic difficulties, thereby hampering on-farm control programmes. Although the prevalence of JD has increased in Ireland, it is not a recognised clinical entity on the majority of Irish farms. The objective of the current study was to complete a longitudinal study in a MAP seropositive dairy herd to investigate the success of culling and calf management in controlling MAP in an Irish context. A secondary objective was to identify if consistent MAP ELISA positive results provided a reliable indicator of JD gross pathology.

Materials and Methods: A 139 cow dairy research herd was recruited to the study. Prior to study commencement, no clinical cases of JD had been reported in the herd. Monthly collection of blood samples for ELISA testing began in May 2012. This continued until December 2014, at

which point sampling continued on a quarterly basis. All samples were tested using the ID Screen Paratuberculosis Indirect Screening Test. Results were reported as S/P%, a positive ELISA reported at readings of greater than 70S/P%. Faecal samples from ELISA positive cows were collected weekly from September to December 2012 and tested by PCR. Faecal samples were also submitted for faecal culture on an intermittent basis. ELISA positive cows were subject to veterinary examinations throughout the testing period.

In 2013, a single cow (Cow A) was selected for pathological examination (PE) on the basis of repeatedly recording ELISA readings greater than 150S/P%. Cow A also yielded multiple positive PCR results and a single faecal culture positive result. In winter 2013 and winter 2015, a number of additional cows were selected for PE (12 in total). In 2013 the selection criteria for culling was a minimum of 6 ELISA positive results over a 12 month period. The final cow was selected based on 2 positive tests and age.

Cows which recorded at least one ELISA positive result were calved in isolation and returned to the herd post-calving. Colostrum from these cows was discarded and their calves fed colostrum from a consistently ELISA negative dam. All other calves only received colostrum from their own dam.

Results: At the first sampling in May 2012, 10 of the 139 cows tested ELISA positive. The highest number of ELISA positives recorded over the testing period was in July 2013, when 16 cows were categorised as positive. These results exclude the three month period after the statutory annual test for bovine tuberculosis. A total of 12 cows yielded greater than six ELISA positive results over the duration of the study. Veterinary examination did not detect any clinical signs of JD in these animals.

Pathological examination of Cow A revealed severe gross changes consistent with JD (July 2013). The remaining cows were chosen for culling based on consistent ELISA positive readings only. None were faecal culture positive. No cow showed gross lesions of JD on PE. Following the culling exercise, 9 cows remained in the herd which had intermittently generated ELISA positive results. Within three months of the final cull, these cows recorded negative ELISA results and remained negative along with the remainder of the herd until August 2015. A total of five cows were categorised as ELISA positive in August. A single cow (Cow B) recorded an S/P% of 254 and, as she was due for culling due to her age, and had previously tested ELISA positive in January 2014, was subjected to PE in November 2015. Clinical veterinary examination prior to culling yielded no abnormalities and no gross lesions were identified on PE. Histological test results are imminent.

Animals reared using JD management protocols are due to enter the milking herd in spring 2016.

Conclusions: Culling programmes when combined with appropriate management have the potential to reduce the herd prevalence of MAP ELISA positive results. Based on the results of this study, consistently positive MAP ELISA results are not a reliable indicator of the presence of pathological lesions associated with JD. Use of additional tests (faecal culture or PCR) is likely to increase confidence in determining cow selection for culling.

Paratuberculosis

P04-004-098

Proteomic analysis of *Mycobacterium avium* subspecies paratuberculosis in vitro and the identification of seroreactive antigens

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Objectives: Johne's disease (JD) is a chronic infectious disease of ruminants. The control of Johne's disease is severely hampered by the lack of sensitive and specific diagnostic tests in the sub-clinical stage of the disease. The causative agent of Johne's disease is *Mycobacterium avium* subspecies paratuberculosis (MAP). We postulate that one route to new diagnostics is through the identification of novel antigens from the pathogen. We aim to achieve this through detailed proteomic analysis of MAP and using these proteins to identify novel seroreactive antigens in sera from infected cattle.

Materials and Methods: In this study, MAP CIT003 was cultured until it reached log phase of the growth cycle at which time it was harvested for analysis by 2-D Gel Electrophoresis. Immunoblot analysis was conducted using pooled serum samples from 9 infected cattle of known Johne's disease status, confirmed using multiple diagnostics. A total of 15 protein spots were identified as seroreactive antigens. Protein spots were extracted and prepared for mass spectrometry analysis using a tryptic digest. Protein identification and bioinformatics analysis was then conducted using Peaks, BLAST and Interpro software.

Results: The MAP K10 proteome was used as a reference proteome for protein identification as MAP CIT003 is at present incompletely characterised. All 15 seroreactive antigens were identified as being present in MAP K10. While some of the antigens have previously been identified as seroreactive (e.g. LprG), 7 were previously uncharacterized and annotated as hypothetical proteins. These latter protein sequences were analysed using BLAST and Interpro to determine similarities in corresponding species of other *Mycobacteria* as well as to identify potential protein functions. A novel seroreactive protein (Q740L1) was predicted to function in response to stress, while another (Q73V64) was predicted to function in metal ion binding. Results suggest potential roles for these novel seroreactive proteins in response to the immune response of the host. The analysis of the function of other identified and previously uncharacterised proteins is currently on-going.

Conclusions: Characterisation of proteins expressed by MAP have the potential to give new insights into the pathogenicity of this important causative agent of disease. Furthermore, the seroreactivity of these proteins may ultimately aid improved diagnosis of JD by means of inclusion into a novel antigen cocktail for JD detection. Confirmation of seroreactivity of identified novel proteins is currently being conducted.

Paratuberculosis

P04-004-099

Presence of *Mycobacterium Avium* Subs. Paratuberculosis Dna in Milk used to Feed Calves in Portugal

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Objectives: Dairy farmers often feed waste milk to calves, milk that cannot be used for human consumption, including colostrum, high cell count milk or milk with antimicrobials from animals under treatment. *Mycobacterium avium* subsp. paratuberculosis (Map), the causal agent of paratuberculosis is transmitted more frequently through the fecal-oral route, with evidence that calves under 6 months of age are the most susceptible to infection. Rearing calves with waste milk may thus increase the risk of transmission of paratuberculosis on farm.

Materials and Methods: This study aimed at detecting the presence of Map on milk fed to calves, and to address possible risk factors for that presence. A questionnaire was performed on 37 commercial dairy farms in Portugal, and waste milk samples were collected on 3 different occasions separated by a minimum of one week. In case of farms not feeding waste milk to calves, bulk tank milk samples were collected instead. A nested real time PCR for the detection of the IS900 sequence was performed for the detection of Map.

Results: A majority of farms (89.2%) fed waste milk to calves. From the 33 farms that fed waste milk to calves, only one pasteurized the milk before feeding it to calves. Results of the IS900 nested real time PCR showed that 51.5% of the farms that were feeding waste milk to calves had a positive result for Map on that milk. None of the studied risk factors were significantly associated with the presence of Map in milk samples, however the risk of having a sample positive for Map on PCR was 3.5 times higher for farms that bought in animals from a single origin and 1.9 times higher for farms that bought from multiple farms, when compared with closed farms. Having a calving area for multiple cows also increased the risk of a positive Map result by 1.5 when compared with single pens for calving. The risk of having a positive Map result on waste milk was 1.6 times higher for farms feeding that milk to male calves and 1.4 for farms feeding to both male and female calves, when compared with farms not feeding waste milk.

Conclusions: This study highlights paratuberculosis as one of the potential risks of feeding waste milk to calves, and the need for mitigations strategies to be in place to avoid unnecessary disease transmission.

Paratuberculosis

P04-004-100

The use of web based economic Health Evaluator to increase farmer engagement with Johne's control

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Objectives: The economic impact of Johne's Disease (JD) is traditionally divided between direct losses (milk loss, culling) and indirect losses and associations (e.g. udder health).

However, JD costs are often expressed as an average cost across all herds, for example, £27/ cow using direct costs only which often underestimate the true impact of JD.

Higher prevalence herds however incur substantively higher costs due to consequential losses of increased JD related culling and retention of cows with low fertility and health potential. The correct estimation of JD costs using farm specific health evaluation can help improve engagement in JD control.

Materials and Methods: In 2007 a Health Evaluator was developed

within a web based Health Planning tool (www.myhealthyherd.com) allowing both the vet and farmer to enter their own targets and costs for all the key health areas (fertility, udder health, lameness, sick cows/metabolic, culling and youngstock). The program was populated with default incidence and cost targets for 29 health values and this allows for farm specific overall losses for health areas to be calculated. The economic benefit between the target for the herd and the actual performance is expressed as a "profit opportunity" which can be achieved by improving performance in that particular area.

The whole herd economic evaluation was combined with outputs from the Disease manager module that used the risk and test prevalence data to predict the estimated future true JD herd prevalence for the herd. The program allowed for an agreed economic evaluation of the estimated impact of diseases and culling losses, which is used to help motivate engagement with veterinary led control programs. The graphical outputs were used within veterinary practices and within commercial and regional control schemes in the UK to help engage over 2000 dairy farmers with effective JD risk assessments with a proportion using health evaluations to support their management plans.

Results: Analysis of 833 UK dairy herds (30,820 culls) within the myhealthyherd program revealed that 23% of all cows culled were culled due to sickness, death or casualty. Within the program the culling reasons and costs are defined and split into losses due to no value culls/deaths (£3,499), emergency culls (£2,182) and culls sold live at the end of lactation (£1,238). The proportion of the herd culled within these categories was further subdivided according to culling reason. 2915 cows were culled due to health problems with a lower, median and upper quartile incidence of 0%, 1.5% and 2.5%. The wide variation between herds clearly illustrates that poor health is not inevitable and can be controlled. The farmer may not be aware of this opportunity unless this is clearly illustrated.

In the authors experience practical on farm health evaluation typically reveals that in herds with high prevalences of Johne's disease the economic impact can be in excess of 1-3 pence per litre due to JD with these losses continuing (at a lower level) for several years before the disease is fully controlled. The economic impact is reflected in consequential losses due to fertility and wider disease issues (due to retention of cows with poor fertility and health potential due to accelerated JD culling). Typically udder health may also be jeopardised as insufficient replacements may be available with penalties for poor milk quality and escalating clinical incidence of mastitis.

Conclusions: The true impact of JD is often underestimated. To fully engage farmers with Johne's control programme a full economic health evaluation can be used to simply illustrate the profit opportunities that can be achieved through effective control. If the audit is performed using agreed targets and costs, which are specific to the farm this will produce a credible figure that the farmer is more likely to comprehend

The use of a farm specific Health Evaluator that illustrates direct, indirect and consequential losses using simple inputs with credible outputs is a useful method of motivating farmers to commit to long term JD control programs.

Paratuberculosis

P04-004-102

The effects of subclinical *M. avium* ssp. paratuberculosis infection on the health and production parameters of dairy cows

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Objectives: Paratuberculosis (also called Johne's disease) is a chronic disease caused by *Mycobacterium avium* ssp. paratuberculosis (MAP). This incurable illness is clinically manifested at 3 or 4 years of age, usually after the 2nd lactation period. It occurs worldwide and it is one of the most economically important infectious diseases of ruminants.

Materials and Methods: The effect of subclinical paratuberculosis infection on the health and production parameters of cows was studied in a Hungarian dairy herd. Fifteen infected (no clinical signs, but faeces PCR positive; MAP+) and 15 healthy (faeces PCR negative; CO) dairy cows were involved in the study. The age of the cows (MAP+: 5.5±1.6, CO: 5.2±2.8 years) and the number of lactations (MAP+: 3.5±1.4, CO: 3.4±2.2) did not differ at the start of the experiment. During the study housing and feeding conditions were the same in both groups.

Blood and urine samples were taken for major biochemical parameters, from 2 weeks prepartum to 120 days postpartum (d -10-14, 2-5, 10, 20, 30, 40, 50, 60, 80, 100, 120) and production data were also collected.

Results: No statistically significant difference was observed between groups regarding blood plasma total protein, albumin, total cholesterol, triglyceride, beta-hydroxybutyrate, non-esterified fatty acid and urea concentrations, and aspartate aminotransferase and gamma-glutamyl transpeptidase activity. Urine net acid-base excretion was higher - but within the physiological range in the infected group. The body condition of infected animals was significantly worse than that of healthy ones, and there was negative correlation between body condition score (-0.53, p<0.0001), the faeces score (-0.37, p<0.0001) and the rumen fill score (-0.22, p<0.001). The milk yield tended to be lower (47.4±12.1 vs. 51.9±13.1, p=0.0632) in the infected group. Other health and reproductive parameters (number of mastitis treatments [2.8±1.3 vs. 0.5±3.2, p=0.002], number of treatments due to reproductive tract problems [6.4±4.6 vs. 2.4±1.8, p=0.0004], length of service period [169.7±70.7 vs. 84.6±33.0, p=0.0001]) were significantly worse in MAP+ cows compared to the control group. First service conception rate was considerably lower in the infected cows.

Conclusions: The subclinically infected cows are more susceptible to other diseases and the latency period of the disease is long, which urges more attention to be paid to the prevention and eradication of paratuberculosis.

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Paratuberculosis

P04-004-103

Associations between Johne's disease and productivity in UK dairy herds

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Objectives: The objective of this study was to quantify the type and magnitude of association between paratuberculosis antibody status and milk yield and fertility parameters in UK dairy cows. A large pre-existing dataset was used to explore associations between MAP antibody status and milk yield and reproductive performance. Quantification of the size of MAP productivity associations in the UK herd was extremely justifiable, given the lack of UK based research and much disparity between production loss estimates from other non-UK studies. This quantification could be further rationalised considering the obvious economic implications of the disease.

Materials and Methods: After data quality analysis; milk recording, fertility and serological data collected from a major milk quality laboratory was retained for 73 herds. Paratuberculosis status was categorised at lactation level via use of the last status per lactation. By definition, each category allocated at the last recording reflected the MAP antibody status of the individual from several preceding measurements. A continuous response multilevel linear regression model was constructed to examine the association between paratuberculosis milk ELISA status and 305 day yield. Two logistic regression models were used to evaluate the association between ELISA status and odds of pregnancy by 100 DIM and ELISA status and odds of service by 80 DIM respectively. Model assumptions and fit were tested and verified in all models.

Results: The following provisional results were derived from this initial analysis. Positive MAP status was associated with a decrease of 366 litres per 305 day lactation compared to lactations with a negative MAP status. A 25% reduction in the odds of service by 80 DIM was also found associated with this status. Furthermore, positive MAP status was associated with a 79% reduction in the odds of pregnancy by 100 DIM. Uncertain MAP status was not significantly associated with 305 day yield and provisionally positive MAP status was not significantly associated with odds of service by 80 DIM.

Conclusions: Positive MAP status clearly has a negative association with milk production in the UK dairy cow. The magnitude of this association however appears less than documented previously, thus an investigation re-evaluating this in the UK dairy herd could be justified. A very large negative association was also observed between positive MAP status and odds of pregnancy by 100 DIM. This provisionally suggests that MAP infection is negatively associated with establishment of pregnancy and to a lesser extent with oestrus expression or post-partum anoestrus. It is important however to note that producer bias may be linked to these negative associations.

Paratuberculosis

P04-004-104

Social network analysis of cattle positive for Johne's disease by faecal culture from submissions to the Regional Veterinary Laboratories in Ireland.

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Objectives: National risk-based surveillance programmes for paratuberculosis (JD) can be hampered by the difficulty in networking disparate databases across organisations. This is particularly important when attempting to track the movement of *Mycobacterium avium*

subspecies paratuberculosis (MAP)-positive animals between herds. This study reports the use of social network analysis (SNA) in the epidemiology of JD. The aim of the study was to describe the network of confirmed (faecal culture) MAP-positive animals in Ireland by linking their movements through different premises.

Materials and Methods: Networks were constructed by linking a dataset of laboratory records of MAP culture-positive animals obtained by passive surveillance over an 11-year period (2005-2015) with the national Animal Identification and Movement System (AIMS) database. The dataset was exported to UCINET v.6 and Pajek32 v.3.01 and organised in an adjacency matrix consisting of a collection of nodes (premises) and an array of arcs (directed movements) linking the nodes (network). Movements to a factory or knackery were excluded and, due to the epidemiology of JD, a movement through a mart from premises A to B was represented as a movement from A to B. The nodes were categorised (attributes) and graphed in an arbitrary space for visual assessment. Networks represent patterns of connectivity of populations and describe aspects of disease transmission. The centrality value (a structural attribute that represents the node's importance within the network) was calculated for each node to determine its relative potential for MAP spread.

Results: In total there were 1062 confirmed (faecal culture) MAP-positive animals with a total of 898 movements. The network was organised in an adjacency matrix consisting of a collection of nodes (n=990 premises; 489 MAP-positive, 501 MAP-unknown status) and an array of directed movements or arcs (n=658) linking the nodes. The network was fragmented into 219 components (sub-networks where nodes are connected within but do not have ties with other sub-networks) ranging from 1 to 81 nodes; 64 (29%) components had two or more MAP-positive herds (nodes) linked by movement of MAP-positive animals. MAP-positive animals were moved once (56.9%), twice (24.4%), thrice (9.8%) or more times (8.9%) between nodes.

Conclusions: Social network analysis (SNA) is a formal methodology for the analysis and illustration of the relationship between movements of animals and transmission of contagious pathogens associated with those movements. This study addresses the relationship between animal movements and disease detection. It identified connectivity between those premises most likely to facilitate the transmission of MAP via animal movements which was not apparent from either the laboratory records or AIMS databases. Thus SNA provides a novel approach to the development of risk-based surveillance for MAP and potentially for other veterinary disease prevention programs.

Paratuberculosis

P04-004-105

Strategic options for the management of Johne's Disease in UK dairy herds

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Objectives: This paper will demonstrate the variety of strategic options used by dairy farmers throughout the United Kingdom for the prevention and control of Johne's Disease in dairy herds, and explain how the most appropriate option can be selected and then tailored to the needs and aspirations of the farmer.

Materials and Methods: Management programmes for the prevention and control of Johne's Disease in dairy herds have been initiated in parts

of the United Kingdom after a major engagement campaign to encourage farmers to determine the risk of Johnes disease to their dairy herds and the current disease status of their herds. Six strategic control options were offered to farmers using a web based herd health management tool (myhealthyherd.com) to help farmers and their veterinary surgeons decide on the most appropriate course of action to manage Johnes Disease in their herds. Once the risks of disease entering the herd and spreading within the herd were established, the current prevalence of Johnes infection was estimated and an appropriate control strategy planned. The risks, prevalence and control option were recorded on a central database and analysed for the purposes of this paper.

Results: Of the herds that were found to be significantly infected with MAP and which selected one of the six options for control, 50% opted to use quarterly milk testing and a risk based control strategy to manage the disease, 20% opted to improve farm management and use single annual testing to identify and manage high risk cows, and 18% chose to improve farm management for all cows without any testing. 8% opted for a traditional test and immediate cull policy. Only 2% decided to vaccinate, and only a few farms used terminal sires and the purchase of replacements to manage the disease.

Conclusions: The study demonstrates that no single management or control strategy suits all dairy herds in all circumstances. Limitations of resources, and differences in aspirations between farms and farmers suggests that different strategic options must be offered for the prevention, control and management of Johnes Disease in dairy herds. The most popular strategic option selected by veterinary surgeons and dairy farmers involved the quarterly testing of all cows and the management of high risk cows to prevent new infections.

Public Health

P02-002-165

Prevalence and characterization of Verocytotoxin-producing *Escherichia coli* (VTEC) in cattle of Northern Italy

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Objectives: VTEC are agents of diarrhea and hemorrhagic colitis in humans, which can result in a hemolytic uremic syndrome (HUS). Serogroups more frequently associated with human infections are O157, O26, O111, O103, O145 (top-5). Ruminants are the natural reservoir of VTEC. The shedding of VTEC in cattle appears to be influenced by age of the animals, season, diet, housing and stress.

Aims of the study were to a) determine the prevalence of VTEC in feces of cattle of different age and attitude of Northern Italy; b) investigate the characteristics of the strains in term of virulence traits (vtx, eae) and serogroups.

Materials and Methods: Fecal samples were collected between June and October 2015 in 43 dairy herds and 19 beef farms of the Lombardy Region. In each dairy herd 6 calves (less than 30 days), 6 heifers and 6 milking cows were individually sampled. In beef farms feces of 6 fattening veals or 6 fattening bulls were collected. Analysis were performed in pools of 3 animals of the same class for a total of 82, 86, 87, 12 and 26 samples respectively in dairy calves, heifers or cows and fattening veals or bulls. Feces were directly plated onto MacConkey agar and enriched in 0.1% peptone water (PW) and plated onto TBX agar. DNA was extracted from PW and positive signals for vtx1, vtx2 and eae genes, as well as for the top-five serogroups were demonstrated with a Real-Time PCR. VTEC isolation from vtx-positive samples was attempted by analyzing 50 colonies from MacConkey agar and from TBX agar plates. Results were expressed as in the ISO13136/2012 as "presumptive presence" in case of positivity for vtx genes without isolation and "presence" in case of successful isolation. 13 serogroups more frequently associated with human infection were searched on isolated strains. Chi-square test was applied to compare the prevalence of VTEC isolation in different classes of animals.

Results: In all the farms the presumptive presence of VTEC was recorded and VTEC were isolated from 53 out of 62 herds, for a total of 157 strains. Overall, the herd prevalence of VTEC (presence confirmed by isolation) was 95.2% (CI95%: 88.8-100%) and 68.4% (CI95%: 47.5-89.3%) in dairy and beef herds, respectively. The genes associated with the top-5 serogroups was frequently detected in fecal samples (in all the herds at least one sample showed a positive PCR signal for at least one of the top-5 serogroup). However, the majority of the isolates did not belong to serogroups commonly found in humans and only 17 strains were positive for the presence of eae and vtx2 or eae, vtx1 and vtx2, gene combinations associated to more severe illness. In 9 herds VTEC of top-five serogroups were isolated, but only in 3 of them the strains were positive for the presence of vtx2 or vtx1 and vtx2 and in particular they belonged to the O157 serogroup.

Prevalence of VTEC (confirmed by isolation) was 42.6% in calves, 60.5% in heifers, 21.8% in cows, 8.3% in fattening veals and 69.2% in fattening bulls. The frequency of VTEC isolation resulted significantly higher in heifers than in calves or cows ($p < 0.05$), as well as in fattening bulls than in fattening veals ($p < 0.01$). VTEC carrying eae gene was isolated in 23

samples from calves, 8 from heifers, 6 from cows, 4 from fattening bulls and no one from fattening veals. The frequency of isolation of VTEC eae-positive was significantly higher in calves than in heifers or cows ($p < 0.01$) and this is mainly associated with the isolation of eae-vtx1 strains.

Conclusions: Differences among class/age of animals in term of VTEC prevalence and strains characteristics were observed. Our results confirm a large presence of VTEC in cattle, but a low presence of strains positive for eae and vtx2 or eae, vtx1 and vtx2 or belonging to the "top five" serogroups, which are more often associated with cases of severe human illness. Genotyping of the strains, including the analysis of additional virulence factors, is in progress and the comparison with human isolates collected in the same area and period will be performed to better understand the possible link with human infections.

Public Health

P02-002-166

Serological survey for porcine cysticercosis and associated risk factors in pigs slaughtered at Ndumbuini abattoir in Nairobi, Kenya

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Objectives: Cysticercosis is a serious public health risk in both rural and urban areas. Eastern and southern Africa has experienced a recent rapid growth in pig production, with increasing number of cysticercosis reports. But Cysticercosis is neglected in Kenya, with scarce data. The objectives of the project were to: estimate the seroprevalence of cysticercosis; map the value chains associated with the abattoir; and assess the risk of cysticercosis-infected pork entering Nairobi food system.

Materials and Methods: This study was conducted at Ndumbuini, the main abattoir supplying pork to middle-low income consumers in Nairobi. Blood samples were obtained from 700 pigs selected by systematic random sampling. Antigen ELISA was used to detect *T. solium* secretory antigen. Focus group discussions and individual interviews were conducted to get information for value chain analysis and understand the risk of cysticercosis-infected pork getting into the Nairobi food system. A total of 60 Pork butcheries in Nairobi were also visited and a structured questionnaire administered to understand how pork is handled at the butchery and the preferred cooking methods.

Results: The overall sero-prevalence of cysticercosis was estimated at 8.5% (95% CI: 6.4-10.7%). Pigs originated from 11 different counties. The prevalence rates ranged between 0-36%. Seven counties had seropositive pigs, four showed no positives. There was significant differences between sex ($p=1$) or age ($p=0.13$) of the pigs sampled. No positive cases were detected by post-mortem meat inspection. Therefore, all the carcasses were passed for human consumption. The abattoir had no facilities for treating *Cysticercus solium* infected carcasses.

Pork is mainly transported from the abattoir at room temperature (97%), majority of retailer (87%) keep pork at room temperature and cysticercosis awareness level is very low (10%).

The highest risk of consuming infected pork from Ndumbuini is in Nairobi County, which receives the largest percentage of pork (69.43%), Kiambu (14.22%), Nakuru (8.87%) and Kajiado (6.59%).

Conclusions: This study highlights that *T. solium* is a public health risk to consumers of pork from Ndumbuini abattoir. We therefore recommend that meat inspection by palpation and incision to be complemented with rapid tests. A multi-sectorial, One Health Approach should be conducted to sensitize communities on control strategies like proper cooking methods, improved food hygiene and sanitation, accompanied with studies to determine the risk factors of *T. solium* transmission in both pigs and people.

Public Health

P02-002-167

Use of mycotoxin binders and aflatoxin M1 detection in raw milk

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Objectives: Aflatoxins are toxic secondary metabolites produced by species of *Aspergillus*. The most extensively investigated aflatoxins are aflatoxin B1 and aflatoxin M₁, because according with the IARC they are carcinogenic and potentially carcinogenic for humans, respectively. Since the AFM₁ is a metabolite of AFB₁ eliminated through milk, AFM₁ levels in milk are an excellent biomarker to AFB₁ consumption.

In order to determine if mycotoxin binders added to feed cattle could reduce AFM1 in field conditions, a descriptive and longitudinal study was performed in three dairy farms.

Materials and Methods: The cattle feed in the dairy farms was naturally contaminated with aflatoxins. Five mycotoxin binders called "broad spectrum binders (bentonite, montmorillonite, aluminumsilicate, clay and glucomanns) were used during 24 months. The dairy farms owners gave their consent to sampling the milk twice a month.

The milk samples were preserved in refrigerated storage and analyzed in The Research Laboratory of Universidad Autonoma de Aguascalientes, Mexico. To determinate AFM1 concentration in milk samples, competitive ELISA kits were used.

Results: The results show that 54.16% of all milk samples had detectable AFM1 levels. No sample exceeded the maximum allowed limit (0.5 mg / L) established by the Norma Oficial Mexicana but 27.31% exceeded the maximum allowed limit (0.05 µg/L) set by European Union.

The results show that binders were effective to maintain the AFM1 levels below of Norma Oficial Mexicana but not for more strict regulations such as the one set by the European Union. These results apply to the three dairy farms. The dairy farms used mycotoxin binder according to labeled directions, showed a tendency to diminish AFM1 level.

Conclusions: According to results, milk or milk products consumers are in potential risk due to AFM1 is not being denaturalized by pasteurization or boiling. None of the process reach a high temperature to destroy AFM1.

Public Health

P02-002-169

Bioaccumulation of Cd and Pb in plant tissues (root, stem and leaves) of grassland in oil producing region of Magdalena Medio, Colombia.

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Objectives: The aim is to estimate the frequency of plants in tropical grassland of the region of Magdalena Medio (Colombia) there are two economic activities: intensive livestock and a consolidated petrochemical industry. These activities thrown into the environment large amounts of toxic metals: such as lead and cadmium, which pollute soils and grassland. We try to establish the relationship between the presence of heavy metals (Cd and Pb) in topsoil, and the response of these plants to the accumulation of heavy metals. Finally we determine the contents of cadmium and lead in different plant tissues settled in the area.

Materials and Methods: It has been done an inventory of soil and pastures to distances of 100, 500, 2500 and 5000 m, from the sources of pollution (oil wells of Yondo-Antioquia, crude oil refinery of Barrancabermeja and Campus of the University La Paz, Barrancabermeja-Santander). Other aspects: such as soil depth (5 to 30 cm), type of grass, part of the plant (root, stem and leaves) and distance from the source of contamination were taken into account.

Plant and soil samples were conducted during the dry season in June 2013. Samples were processed by atomic absorption spectrophotometer (Varian Spectra AA 20FS) used for the determination of metals, equipped with a lamp hollow cathode appropriate for each element, and a deuterium lamp for background correction.

Results: Seven species of *Brachiaria* have been detected. They are tropical grasses adapted to soils with heavy metals such as Cd and Pb. In the area of refinery B. decumbens were detected, alternating with B. humidicola and B. brizantha. In the area of oil wells and the area control they presented more often other species. Both the distance to the emitting source, such as the sampled area showed a statistically significant effect on the bioaccumulation of Pb. This metal bioaccumulates at very high concentrations, preferably in the roots, and then the stems.

In coming to the oil refinery fields and in transects of 100 and 500 m, it was where the greatest gradients of Pb contamination occurred. In the case of Cd on stalks as bioconcentration it involves both refining zones and in oil wells, and especially in the roots. In the bioaccumulation of Cd no noticeable decrease due to the distance to the emitting source.

Conclusions: It has demonstrated the adaptation of *Brachiaria* to soils with high persistence of heavy metals, especially in their first 5 cm, with great bioaccumulation of Cd and Pb, and especially their root systems.

Public Health

P02-002-170

Selective Androgen Receptor Modulators (Sarms): Zootechnical Effects And Analytical Strategies To Detect Their Administration In Calves

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Objectives: Selective androgen receptor modulators (SARMs) are a novel class of androgen receptor ligands. They are intended to exhibit the same kind of effect as androgenic drugs but be much more selective in their action. They will also have a high potential for misuse in breeding practice. In order to guaranty the consumers with food from animal origin free from any residues, studies about SARMs and analytical strategies are required to ensure safe food. Two objectives will be carried out: 1- to investigate SARMs zootechnical performances in calves, 2- to study SARMs kinetic of elimination and metabolism in calves urine and feces.

Materials and Methods: An animal trial was carried on under approval of the French Ethical Committee at the Center of Research and Preclinical Investigation (CRIP of Oniris). Twelve Prim'Holstein entire male weaned calves were purchased to establish a protocol for assessing the repeatability of seven parameters to allow describing and quantifying their growth and development (live weight gain, growth rate, intake efficiency, width of hips, girth circumference, pelvis muscle depth, size and Body Condition Score). Five measurements per parameters were realized/repeated. All animals were followed clinically every day, urines and feces collected by the same operator. Among these twelve calves, ten of them have been selected to create a "treated" group (n = 5) and a "control" group (n = 5) to study the zootechnical effects after an oral administration of enobosarm. In the same time, the kinetic of enobosarm elimination was established and the metabolism investigated after oral administration at either a concentration of 1 mg/kg (1 ppm "low dose") or with a calf orally "treated" once with 200 mg on one-shot ("high dose"). Liquid chromatography hyphenated to a tandem mass spectrometry (LC-MS/MS) was conducted using a ultra-high pressure liquid chromatography (UHPLC) on reversed-phase separation equipped with an Acquity UPLC BEH C18 column and connected with a mass spectrometry detection in electrospray ionization (ESI) negative mode with a triple quadrupole (QqQ) Xevo TQ-S.

Results: Beside the physiological growth of calves, measures of body weight (BW), growth rate (ADG) and feed efficiency (FC) showed no significant difference in growth between treated and control groups. Similarly, no significant stature growth difference between treated group and control group could be observed on others measurements performed. In urine and feces, the drug could be identified very shortly after oral administration. At the "high dose", the urine concentration of free form of enobosarm was between 0,24 and 38 ng/mL with a maximum at D+5H. After 5 days, enobosarm could not be detected anymore. The concentration of the total forms of enobosarm was detected between 0,25 and 155 ng/mL with a maximum at D1. After deconjugation, enobosarm could be detected with a low level until nine days. During the 21 days of treatment at the "low dose" the average level of free form of enobosarm was between 0,18 and 1,2 ng/mL. And the total forms of enobosarm was detected with an average concentration between 0,4 and 2,8 ng/mL. In feces, with the "high dose", only free form of enobosarm was always and very comfortably identifiable and quantifiable during the 21 days of the experiment. Concentrations ranging from about 50 000 ng/g to 20 ng/g never dropped below the limits of detection and quantification. With the "low dose", the free form of enobosarm was also identifiable and quantifiable in feces throughout the 21 days of the experiment. The fecal

concentration of enobosarm increased the first three days up to 1800 ng/g and then stabilized from D4 to a plateau of about 1200 ng/g.

Conclusions: Results obtained by analytical strategies allowed to demonstrate a strong fecal excretion of enobosarm, the concentrations are higher in the feces compared to urine samples. Such results are particularly promising in the objective of setting up an analytical strategy and a target matrix to highlight SARMs abuse in livestock animals. Next step is to develop an analytical strategy with a LC-MS based metabolomics approach to allow profiling biological matrices, to reveal biological effects for implementing a robust statistical model to predict illegal use of this novel class of anabolic agents in calves.

Public Health

P02-002-171

Awareness, Attitude and Practices towards Brucellosis among the community in Kiptome location, Kericho District, Kenya

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Objectives:

1. To determine the awareness of brucellosis among the community members within the study area
2. To determine the attitude of the community members towards brucellosis within the study area
3. To determine the practices of the community members towards brucellosis within the study area

Materials and Methods: This study was conducted among the household heads in Kiptome Location, Kericho District, Kenya. Descriptive cross sectional study design was employed. A systematic random sampling method was used to identify 423 household heads for the study. Information on personal data, awareness, attitude and practices towards management of brucellosis was collected using a questionnaire. The data was analysed using Statistical Package for Social Sciences (SPSS) version 17. Ninety five percent confidence intervals (95% CI) and two-tailed p-value was calculated to assess significance of results.

Results: The results showed that (303) 71.6% of the respondents had adequate awareness of brucellosis. There was a high rate of 'positive' attitude towards brucellosis with (315) 74.5% of the respondents having positive attitude and (264) 64.8% of the respondents had good practices in regard to the management of brucellosis. Level of education positively affected awareness, attitude and practice of the community towards brucellosis (p< 0.05). Primary level of education was significantly associated with good practice with respect to brucellosis (p=0.005).

Conclusions: There was adequate awareness of brucellosis, high rate of positive attitude and good practices towards the management of brucellosis among the respondents. The non-sanitary practices like the lack of use of protective devises while handling aborted fetuses and the non-use of parturition pens for livestock, in regard to the control of brucellosis can predispose the community members to the disease. This study recommends that education and awareness of brucellosis should particularly focus on the improvement of the use of parturition pens and handling of aborted fetuses and other materials from pregnant animals.

Reproduction

P02-002-172

Comparison Between Techniques By Per Rectal Palpation For Pregnancy Diagnosis On Pregnancy Loss, Calving Rates And Abnormalities In Newborn Calves

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Objectives: The objectives of the present investigation were to evaluate the pregnancy diagnosis by detection of either the allantochorion membrane (FMS) or amniotic sac (ASP) by per rectum palpation (PRP) during late embryonic or early fetal period on pregnancy loss (PRL) at reexamination, calving rates, and abnormalities in newborn calves

Materials and Methods: A controlled randomized blind design with 800 lactating dairy pregnant cows diagnosed by transrectal ultrasonography (TRUS) between days 35 and 57 of gestation from one dairy farm were included. The cows were randomly divided according to detection of allantochorion membrane (FMS group; n= 264), detection of amniotic sac (ASP group; n= 266), and TRUS (control [CON] group; n=270). TRUS was considered as the criterion standard method of comparison. The entire PRP was performed by one experienced veterinarian. Then, all the cows were reexamined only by TRUS between 2 and 4 weeks later by two independent veterinarians to assess PRL. The calving rate one (number of cows calved/number of cows initially pregnant) and calving rate two (number of cows calved/number of cows pregnant at reexamination) for each group was calculated. All abortions and stillborn were necropsied, and calves alive were followed for 5 days.

Results: The overall initial PRL (between initial pregnant cows and reexamination) for FMS, ASP, and CON groups was 7.4% (19/258), 8.8% (23/262), and 9.2% (24/260), respectively (P=0.75). The number of missing animals between initial pregnancy diagnosis and reexamination from groups FMS, ASP, and CON was 6, 4, and 10, respectively (P=0.25). The overall late PRL (between reexamination and calving) for FMS, ASP, and CON groups was 4.2% (9/213), 5.7% (12/209), and 4.2% (9/216), respectively (P=0.71). The number of missing animals from reexamination to calving from groups FMS, ASP, and CON was 26, 30, and 20, respectively (P=0.43). The calving rate one for FMS, ASP, and TRUS groups was 79.1% (204/258), 75.2% (197/262), and 79.6% (207/260), respectively (P=0.63). The calving rate two for the same groups was 85.4% (204/239), 82.4% (197/239), and 87.7% (207/236), respectively (P=0.27). The number of fetuses aborted late, premature, and mature dead from FMS, ASP and CON groups was 6, 4, and 5, respectively (P=0.85), and no abnormalities at necropsy were detected. One stillborn male calf with atresia coli after 281 d of gestation from a cow examined by ASP at day 51 was diagnosed.

Conclusions: It was concluded that the use of either FMS or ASP for pregnancy diagnosis during late embryonic or early fetal period did not increase the PRL, affect calving rates, or produce calves with congenital abnormalities.

Reproduction

P02-002-173

Effect on pregnancy rate and association between CL and follicle sizes and fertility in beef cows subjected to a timed AI protocol, Shortsynch

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Objectives: Although prostaglandin F2a (PG) has been widely used for induction of estrus in beef as well as dairy cows, treated animals are often not inseminated because induced estrus is not detected. Shortsynch is a protocol that includes injection of PG to the animals having a corpus luteum (CL) (day 0) and GnRH injection 48-56 h later (day 2) followed by timed artificial insemination (TAI) 16-20 h later (day 3). The objective of the study was to elucidate effect of Shortsynch on pregnancy rate (PR = CR x estrus detection rate) and association between CL and follicle sizes and conception rate (CR) in Japanese Black cows subjected to the protocol.

Materials and Methods: Experiment 1: A total of 272 postpartum Japanese Black cows having a CL 18 mm or greater in diameter were randomly divided into two groups; those subjected to Shortsynch and TAI (SSTAI, n = 134) and those injected with PG and inseminated after estrus detection (PGAI, n = 138). Size of CL was evaluated by ultrasonography with a linear probe of 10.0 MHz. Pregnancy diagnosis was made by ultrasonography 30 to 50 days after AI. CR and PR were compared between the two groups. Experiment 2: A total of 38 postpartum Japanese Black cows having a CL 18 mm or greater in diameter were subjected to Shortsynch and TAI. Diameters (average of the major and minor axes) of CL and the largest follicle and volume calculated by a formula for ellipsoid volume were recorded and plasma samples were collected on days 0, 2 and 9. Plasma progesterone concentrations were determined with a validated enzyme-linked fluorescent assay (miniVIDAS; bioMerieux, Marcy l'Etoile, France). CL and follicular sizes and progesterone levels were compared between subsequently pregnant and non-pregnant cows. Receiver operating curve was used to identify the optimal cutoff point of CL size for fertility.

Results: Experiment 1: While no difference was found in CR between SSTAI (81/134, 60.4%) and PGAI (71/120, 59.2%), PR in SSTAI (60.4%) tended to be higher than that in PGAI (71/120, 51.4%) (P = 0.09). Experiment 2: Progesterone concentrations on day 0 were 9.7 ± 3.0 ng/ml, and higher than 1.0 ng/ml in all of the animals. CR (PR) was 60.5% (23/38). Diameter of CL on day 0 in pregnant cows (20.8 ± 1.4 mm) was greater (P < 0.05) than that in non-pregnant cows (19.8 ± 1.6 mm). Volume of CL on day 0 in pregnant cows (4.11 ± 0.77 cm³) was also greater (P < 0.01) than that in non-pregnant cows (3.24 ± 0.80 cm³). The optimal cutoff points of CL diameter and CL volume for fertility were 19.8 mm and 3.61 cm³, respectively. Diameter of the largest follicle in pregnant cows (10.0 ± 2.5 mm) was similar to that in non-pregnant cows (9.1 ± 1.7 mm) on day 0. On day 2, however, the diameter in pregnant cows (12.4 ± 1.8 mm) was larger (P < 0.05) than that in non-pregnant cows (11.1 ± 2.1 mm). Progesterone concentrations on day 9 in pregnant cows were higher (P < 0.05) than those in non-pregnant cows.

Conclusions: In conclusion, application of Shortsynch is effective in improving PR, and use of ultrasonography for accurate evaluation of CL and follicle sizes during the protocol process could help to increase CR in cows with a functional CL.

Reproduction

P02-002-174

Detection of bovine leukemia virus antibodies in receptive cows

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Objectives: The causative agent of enzootic bovine leukosis (EBL) is the Bovine leukemia virus (BLV) a retrovirus that infects beef and dairy cattle. It is a major animal health problem worldwide causing important economic losses and this agent is able to cross transplacental barrier. Enzootic bovine leukosis can lead to losses as inability to export cattle, semen and embryos to countries that maintain BLV control programs. Further losses may include reduced reproductive efficiency and decreased milk production. The aim of this study was to select bovine receptive for embryo BLV negatives.

Materials and Methods: The serum samples were of receptive cows from an embryo transfer program in order to establish performance standards for the embryo transfer industry to ensure the accuracy and completeness of all records pertaining to the parentage of resulting offspring. A total of 3895 serum samples of cows above 24 months, mixed breed cattle, were analyzed with the aim to select the BLV negative for the program. Samples were analyzed by AGID in accordance to OIE, at Bovid Viruses Laboratory of Instituto Biológico, São Paulo.

Results: The frequency of BLV positives by AGID were 5,8% (226/3895). None of the animals showed clinical signs indicating that were asymptomatic carriers of the virus. Only BLV negatives cows were selected to embryo transfer program. In Brazil, the frequency of positive animals varies considerably between the states considering the breeding, dairy or beef cattle, and management. Sero-epidemiological surveys have been demonstrated rates between 9,0% and 54%, showing that BLV is widespread and measures to contain the infection need to be taken.

Conclusions: The sanitary control for BLV in embryo transfer program is important to mitigate the risk of spread and prevent the cycle of transmission of disease by transplacental and horizontal vials. So, to avoid losses caused by infection.

Reproduction

P02-002-176

Comparison of Carotenoid Contents in the Blood and Follicular Fluid of Japanese Brown Cattle During and After the Grazing Period

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Objectives: Carotenoids are important nutrients not only for humans, but also for animals. Carotenoids enhance immunity and reproductive

performance for livestock. Fat soluble vitamins and β -carotene enhance bovine ovarian function, follicles and embryos. Carotenoids show excellent singlet oxygen quenching activity, and have a protective effect against UV light. However the function of carotenoids in cattle is not clear enough. The aim of the present study was to investigate comparison of carotenoid contents during and after the grazing period.

Materials and Methods: Three Japanese Brown cattle, which were born on the farm of the Faculty of Agriculture at Tokai University, were used in this study. The test period was divided into two periods, based on the grazing seasons (During: during the grazing season in October of 2014, n=5; After: after the grazing season in November of 2014, n=6). Cattle were fed 10 kg of green grass dry matter (DM) per day per head on grasslands in the Aso region during the grazing period, and 8 kg of silage DM per day per head in the barn after the grazing period. An investigation into the accumulation of carotenoids from feed was carried out. The accumulated carotenoid contents at the estrous period in the blood and follicular fluid were observed. Blood estradiol level (E_2) and progesterone level (P_4) was measured.

Results: Among the accumulated carotenoid contents in the blood and follicular fluid at the estrous period, β -carotene was presented as a major component along with lutein. In the blood, the lower β -carotene level in the After period was observed as (918.7 ± 290.3 ng/mL) compared with that in the During period as (4325.4 ± 1000.9 ng/mL, $P < 0.01$). In addition a lower lutein level was observed in the After period as (15.1 ± 7.6 ng/mL) compared with that in the During period as (189.0 ± 41.7 ng/mL, $P < 0.01$). Although there is no statistical significance, a lower β -carotene level of the follicular fluid was observed in the After period as (968.5 ng/mL \pm 162.8 ng/mL) compared with in the During period as (1493.0 ng/mL \pm 681.3 ng/mL, $P = 0.10$). However a lower lutein level was observed in the After period as (11.4 ± 5.7 ng/mL) compared with that in the During period as (63.9 ± 40.2 ng/mL, $P < 0.05$). Moreover in estimating follicular accumulated concentration, a lower lutein level in the After period was calculated compared with that in the During period (16.0 ± 13.2 μ g vs. 77.2 ± 62.5 μ g, $P < 0.05$). The β -carotene concentration included in the silage is one-seventh to one-twelfth that of fresh green grass and the lutein concentration was one-fifth to one-tenth. Moreover a small amount of β -cryptoxanthin was detected in the corn, and a small amount of zeaxanthin was detected in the silage. Although there was no difference in blood E_2 and P_4 levels between the two periods, β -carotene and lutein levels were lower after the grazing period than during it.

Conclusions: These studies indicate that carotenoids concentration of blood and follicular fluid is dependent on the carotenoids concentration in the feed. It was found that in the grazing periods (from April to October) grazing cattle took in carotenoids (β -carotene 2g/day, lutein 0.5g/day). The ideal calving season in Japan is in April. Cattle which deliver in April can eat a sufficient amount of fresh green grass for six months. Carotenoids might be especially necessary for bovine calving, milk secretion, conception and maintaining pregnancy. Therefore more carotenoids might be necessary for breeding cattle which cannot be fed enough fresh green grass.

Reproduction

P02-002-177

Bovine Obstetric Events attended by New Zealand Veterinarians and Risk Factors Associated with Method of Calf Delivery

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Objectives: Approximately 4.8 million dairy cows calve annually in New Zealand (NZ). Anecdotally, veterinarians are called to assist in a significant number of these calving events. To date little data has been published on neither the types of dystocia for which veterinary assistance is sought, nor the treatment protocols chosen by veterinarians. The objectives of this study were to:

1. Describe, at a national level, the relative frequency of different types of dystocia for which veterinary assistance is sought.
2. Describe the relative frequency of, and risk factors associated with, the use of each type of calf delivery method by veterinarians in NZ.

Materials and Methods: Data on calving and uterine prolapse events was entered on a voluntary basis by veterinarians (vet) into an online reporting system (www.calvingcup.bayer.co.nz) from the 10th June 2014 to the 31st October 2014.

A calving event occurred if one or more calves were delivered by the vet. The vet recorded the obstetric situation they were presented with, the method used to deliver the calf, the date, and the regional location of the event. The country and year of vet graduation, and whether that vet was a new graduate were also listed. The number and percentage of vets or events, by each risk factor, were described.

Each method of delivery (correction/traction, foetotomy or caesarean) was dichotomized into whether the method was used or not used, with a separate model run for each outcome. Non-normally distributed risk factors were summarized using the mean and standard deviation. Risk factors were screened using a t-test, Chi-squared test, or Fisher's exact test and those associated ($p < 0.2$) were included in multivariable regression analysis.

A backwards stepwise model-building process was used to develop mixed effect logistic regression models, with vet included as a random effect. Variables were retained if they were associated with $p < 0.05$, derived from a likelihood-ratio test. Biologically plausible interaction terms between main-effects variables were considered for inclusion in each model.

Results: 9792 events were recorded as a "calving" by 371 dairy vets. The most common causes of veterinary attendance were a foetal head or leg(s) back, foeto-pelvic disproportion, and lack of vaginal/cervical dilatation +/- uterine inertia. Foeto-pelvic disproportion and maternal pelvic deformity/fracture occurred significantly later in the season compared to foetal head back presentation. Over 80% of cases of posterior presentation, hydroallantois/hydrops, multiple pregnancy, failure of cervical/vaginal dilatation +/- uterine inertia, breech presentation, or twisted uterus were rectified via correction of malpresentation +/- traction. The presentations for which caesarean section was most commonly used were shistosoma reflexus (caesareans performed in 20.5% of events), maternal pelvic deformity/fracture (19.6%) or foeto-pelvic disproportion (12.4%). Relative to foetal head back, (the most common dystocia recorded), shistosoma reflexus (OR = 30.64), maternal pelvic deformity/fracture (OR = 30.18), foeto-pelvic disproportion (OR = 17.67), and twisted uterus (OR = 15.70) were significantly more likely to be delivered via caesarean. Foetotomies were significantly more likely to be performed in the South Island regions (OR = 2.54 - 3.86) relative to the reference category of the Waikato/Bay of Plenty Region (Central North Island). Relative to new graduates, more experienced vets (except those graduated prior to 1979 and between 1990-1994), were significantly more likely to perform a caesarean (OR = 1.66 - 2.80).

Conclusions: This report describes a substantial dataset on veterinary assisted dystocia events in NZ. The type of dystocia, regional location, the country of the vet's training, and the vet's level of experience were

all significantly associated with which method was chosen to deliver the calf. This study also introduces a novel on-line technique for collecting large volumes of clinical data.

Reproduction

P02-002-178

Comparative global gene expression profile of developmental related genes in parthenogenetic and in-vitro fertilized caprine embryos

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Objectives: To examine the global gene expression profile to search differentially expressed genes between parthenogenetic and in-vitro fertilized (IVF) morula by using microarray analysis and validated by relative real time PCR (qPCR) in caprine species

Materials and Methods: Production of parthenogenetic and IVF embryos.

Parthenogenetic and IVF embryos were produced as per the method described by Singh et al, (2013).

Microarray analysis

For microarray analysis, diploid parthenogenetic and IVF embryos of morula (50 pooled embryos) stage were picked up from the culture medium separately and washed with 1X PBS. Two replicates of each group were taken for microarray study. Microarray data were normalized across arrays to perform direct comparisons of probe set values between samples. GeneSpring GX 11.5 microarray data analysis tool was used to identify differentially expressed transcripts. For a given comparison, IVF derived embryos was selected as the baseline reference, and transcripts that exhibited various fold change relative to the baseline were considered differentially expressed. The unpaired t-test method was applied and p-value cut off 0.05 was considered statistically significant. Some of the genes were validated using real time PCR. For comparison of gene expression of diploid parthenogenetic embryos with IVF embryos, the relative expression of data was analyzed using SPSS software and P-value < 0.05 was considered significant.

Results: According to the microarray analysis, the total number of up regulated (UR) and downregulated (DR) genes was 613 and 220, respectively in diploid parthenogenetic morula as compared with IVF morula. The number of genes showing about two-, two- to five-, five- to 10-, 10- to 20-, and above 20-fold UR and DR genes was 147, 229, 122, 59, and 56 and 94, 73, 18, 13, and 22, respectively. Five UR genes validated (PTEN, PHF3, CTNNB1, SELK, and NPDC1) and all of them were significantly higher in parthenotes, which was in accordance with microarray results, whereas the expression of DR (AURKC and KLF15) genes were downregulated in parthenotes as observed in microarray results but the difference was not significant ($P < 0.05$).

Conclusions: In conclusion, our findings demonstrate differential expression of a large number of genes in parthenotes compared with IVF embryos, which may be the reason for aberrant parthenogenetic embryo development in caprine species.

Reference: Singh, R., Kumar, K., Ranjan, R., Kumar, M., Yasotha, T., Singh, R.K., Das, B.C., Sarkar, M., and Bag, S., (2013). *Zygote* 1-7. DOI: <http://dx.doi.org/10.1017/S096719941300049X>

Reproduction

P02-002-179

Absence of association between pre and post partum energy status and genital tract inflammation in dairy cows

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Objectives: Inflammation of the genital tract, i.e. persistent inflammation of the uterus and/or the cervix is one major limiting factors of reproduction in dairy cows. Negative energy balance, both pre- and post-partum, belongs to the main risk factors for genital inflammation. Its early diagnosis could allow at the individual level, an early detection of at-risk cows together with, at the herd level, the evaluation of the quality of the transition period management. The objective of the study was to evaluate the value of several markers of negative energy balance, for the prediction of genital inflammation.

Materials and Methods: Markers of energy balance were selected based on their applicability whereas genital tract inflammation was diagnosed and quantified using uterine and cervical cytology. Samples and data were collected over a 3-months period from 133 cows from 10 herds (Brittany, France). 14 to 3 days before calving, body condition was scored (0-5 scale; BCS) and blood was collected on dry tubes from the coccygeal vein for non esterified fatty acids (NEFA) measurement. Between 3-14 days after calving, BCS and blood (NEFA measurement) were collected and beta hydroxybutyrate (BHB) assayed with a cow-side strip test (Optium Xceed, Abbott, Rungis, France). Blood was stored at -4°C until centrifugation, and serum frozen at -20°C until analysis. NEFA were assayed by spectrophotometry (Diaglobal, Berlin, Germany). With this method, the blood NEFA concentration threshold for genital health is 0.17 mmol/l before calving, 0.35 mmol/L 3-14 days after calving. For BHB, the genital health threshold is 1.1 mmol/l (Dubuc et al, 2010).

At 21-35 d after calving, BCS and vaginal content were collected. Endometrial and cervical smears were performed (cells being collected with a cytobrush). After smear staining (555 staining, RAL, Libourne, France), the proportion of neutrophils >200 cells was counted. Cows with more than 6% neutrophils on uterine smears were considered affected by endometritis, whereas those with more than 5% on cervical smears were defined as a cervicitis. A cow was defined affected by genital inflammation when the uterus and/or the cervix was found inflamed.

Results: Prevalence of cytological cervicitis was 23%, and 47% for endometritis. In total, 53% of the cows were affected by genital inflammation, but only 17% had both compartments simultaneously inflamed. Median body condition score was 3.5 [2.5-4.5] [min-max] before calving, 2.75 [2-4] at 3-14 days after calving and 2.5 [1.5-3.5] at 21-35 days after calving. NEFA blood concentration was above threshold in 40% of the cows before calving and in 50% of the cows at 3-14 days after calving. BHB was above the threshold in 51% of the cows.

No correlation was found between the proportion of cervical or uterine neutrophils and any risk factor tested (BCS before and after calving, BCS variation, NEFA concentration after and after calving, variation in NEFA concentration, BHB concentration). From a qualitative point of view (cow affected or not), the presence of inflammation was not associated with any of the tested risk factors, neither for cervicitis, nor for endometritis or genital inflammation.

Conclusions: BCS, BHB and NEFA concentrations were not associated with the risk of cervical, uterine or genital inflammation and not linked

to inflammation severity. Our results suggest the impairment of reproduction associated with negative energy balance is not mediated by an inadequate uterine microenvironment. One alternative hypothesis is the alteration of oocyte quality.

At the herd level, showing negative energy balance allows to correct transition feeding; when a cow is identified as having a severe negative energy balance, further research is needed to evaluate the impact of a corrective supplementation (e.g. propylene glycol) on reproduction.

Reproduction

P02-002-180

Leptine, Transferrine and α 1-Acid glycoprotein correlations in goats fed with different fat sources and submitted to laparoscopic ovum pick-up

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Objectives: The aim of the present study was evaluate the acute phase proteins (APP) profile and their correlation with plasma leptin levels in eighteen goats fed with different fat sources and submitted to twice-laparoscopic ovum pick-up (LOPU).

Materials and Methods: Eighteen Anglo-Nubian goats were randomly divided into 3 groups based on their diets with different fat sources [soy oil (S, n=6), linseed (L, n=6) and Megalac® (M, n=6)], formulated with 40% concentrate, 60% corn silage and 4% ether extract (in dry matter) from the fat source. The animals were submitted to 15 days adaptation period and, then, an experimental period of 77 days. The LOPU was performed after 36 hours of an ovarian superstimulation protocol [FSH (80mg) + eCG (300IU)], on Day 42 (LOPU1) and Day 70 (LOPU2) of the trial. The blood samples were taken just before each LOPU (D42 and D70), 24 hours (D43 and D71), and seven days (D77) after the second LOPU. The fraction of proteins were obtained by SDS-PAGE and leptin levels were obtained by radio immune assay. Data were analyzed by ANOVA for repeated measures with Tukey post-hoc test ($p \leq 0.05$) and the correlations by Pearson correlation test using software R. There were no interaction between groups and time; furthermore, the results are expressed in principal components by means \pm sd with capitl letters denoting statistical difference.

Results: Were found between 16 to 18 proteins and for this study transferrine (TRANS, mg/dL) and α 1-Acid glycoprotein (α 1-AGP, mg/dL) were elected as relevant. Leptine was similar between groups (S (2.02 \pm 0.83), L (1.85 \pm 0.96), M (2.21 \pm 0.84)) and the mean of D42, D43, D70, D71 and D77 are 1.92 \pm 0.78ABC; 1.86 \pm 0.86BC; 1.54 \pm 0.55C; 2.28 \pm 0.77AB; 2.53 \pm 1.13A. TRANS means between groups are S (467.87 \pm 184.50); L (600.61 \pm 139.23) and M (548.93 \pm 171.62) and the mean of D42, D43, D70, D71 and D77 are respectively 489.06 \pm 176.91B; 476.88 \pm 127.5B; 574.83 \pm 202.49AB; 527.53 \pm 194.22AB and 627.38 \pm 119.95A. α 1-AGP means between groups are S (47.17 \pm 28.90); L (54.95 \pm 30.31) and M (53.09 \pm 33.52) and the mean of D42, D43, D70, D71 and D77 are respectively 54.71 \pm 38.53 ; 46.39 \pm 24.50; 53.48 \pm 31.69; 57.12 \pm 35.80 and 46.96 \pm 22.35. Both TRANS and α 1-AGP were positively correlated with leptine (R=0.32, $p=0.002$ and R=0.21, $p=0.04$; respectively) and positively correlatated with each other (R=0.28, $p=0.006$). TRANS and α 1-AGP was similar between groups and only TRANS demonstrate

moment effect with highest value of TRANS detected on D77 and the lowest on D43. TRANS is related with iron metabolism and a negative APP (decrease the serum values when a inflammatory process installs) in this study were noticed that serum levels of TRANS increased seven days after the second LOPU (D77). The lowest value of leptine were detected on D70 and highest on D77 (similar to TRANS).

Conclusions: According to these results Leptine, TRANS and α 1-AGP were not affected by fat source; despite what was reported in monogastric animals and humans the enrichment with polyunsaturated fat acids (PUFA). Leptine can be described as an adipocyte-derived cytokine and our results shows a low correlation with TRANS and α 1-AGP that should be more investigated.

Reproduction

P02-002-181

The effects of varying levels of dietary starch on reproductive traits in lactating dairy cows

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Objectives: The aim of this study was to determine the effects of different dietary starch levels on a) postpartum ovarian follicular development, b) first postpartum ovulation day, c) involution of the genital tract, d) ovulation time and ovulatory follicle size after induced estrus, e) luteal size after ovulation, and f) the increase in progesterone levels 7 d after ovulation.

Materials and Methods: A total of 23 primiparous Holstein - Friesian cows were used in this study. They were fed three different diets that included different starch levels in milk on a dry matter basis from parturition until 80 d. Cows were randomly assigned into the three groups according to the starch levels of the diets: low (16%, LS group, n = 7), medium (20%, MS group, n = 8), and high (24%, HS group, n = 8). Milk yields were recorded daily, and body conditions (BCS) were scored every week. Transrectal ultrasonography was performed, and blood samples were taken three times each week to determine reproductive traits and blood metabolites.

Results: Although the milk yield was greater in the LS group compared to the other groups, BCS and blood metabolites did not differ between the groups. The mean size of the largest follicle during the first week postpartum (6 ± 1 d postpartum) was 9.6 ± 0.5 mm; this did not differ significantly (P > 0.05) among the groups. The times to the emergence of the first follicular wave after parturition (8.83 ± 1.0 d, 9.37 ± 0.95 d and 10.12 ± 0.95 d in the LS, MS, and HS groups, respectively) did not differ significantly (P > 0.05) between the groups. The postpartum follicular patterns, times of first postpartum ovulation, and involution processes did not differ among the groups. In addition, the ovulatory follicle and corpus luteum sizes and the progesterone levels after induced estrus were found to be numerically greater in cows fed with high starch levels than in other cows.

Conclusions: In this study, different dietary starch levels did not affect blood metabolites or early or late postpartum reproductive traits. However, milk yield was found to be higher in the LS group than in other groups

Reproduction

P02-002-182

Equine Chorionic Gonadotropin Use in Fixed-Time Artificial Insemination Protocols in Non-Cyclic Cows

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Objectives: In Brazil, about 11% of the 65.5 million female cattle, eligible for breeding, are artificially inseminated. 70% of them have zebu genetics and are beef breeds. Usually, estrus detection is difficult and zebu females show a long postpartum anestrus. Beef cattle are raised extensively, making conventional insemination impossible. Hormonal protocols allowing Fixed-Time Artificial Insemination (FTAI), even in acyclic cows, have widely spread. The purpose of this study was to determine the efficacy of equine Chorionic Gonadotropin (eCG) in accelerating follicular growth rate, and in improving ovulation rate in a FTAI protocol in acyclic cows.

Materials and Methods: Forty crossbred cows were used (20 cows and 20 heifers) with an absence of a corpus luteum and the presence of at least one ≥7mm diameter follicle. The animals were submitted to the following protocol: D0: placing a progesterone implant and application of 2 mg estradiol benzoate; D8: removal of the implant and IM application of 0.5mg sodium cloprostenol (Ciosin® MSD Saúde Animal - Brazil). On this day, the ovaries were evaluated and the diameter of the largest follicle was measured. According to the diameter, females were divided evenly into two treatments, via IM, in D8 yet. T1 (n=20): 300 IU of eCG (Folligon® - MSD Animal Health - Brazil) T2 (n=20): 1.5 mL of saline. On D9, all animals received 1 mg of estradiol benzoate, via IM. Ultrasound was used (Mindray-M5) with 7.5 Mhz linear transducer for ovaries evaluation. Tests for detecting the largest follicle diameter were performed from D8 to D10 every 24 hours. From D11 to D12 the ovulation was observed, which was confirmed on D17. The average follicular diameter of each treatment on each day was compared by the Tukey test. The percentage of ovulation were compared using Fisher's exact test. Probability lower than 5% was considered significant. For analyzes it was used the SAS software version 9.3.

Results: In both categories alone (cows and heifers) and in all females together there were no differences (P>0.05) in the average follicular diameter at D8, i.e. on the day the animals were distributed within their treatments. This lack of difference shows that the animals were equally randomized in treatments and there was no effect of follicular size prior to the treatment. There was an increase in the rate of follicular development in animals treated with Folligon®. In cows under this treatment, the follicular development was faster, showing the tested product activity. The mean follicular diameter was as follows. D8: 8.9+2.3 vs. 9.0+3.1; D9: 10.0+3.7 vs. 9.2+3.4; D10: 11.0+2.7 vs. 9.1+4.1 for T1 and T2, respectively. In D9, 24 hours after product administration, there was a difference trend (P=0.09) among the average diameter. The next day, i.e., D10, the difference between treatments was significant (P<0.05). The percentage of cows that ovulated at the end of protocol between D11 and D12 was higher than T1 (95.0 vs. 30.0% - P<0.05). The number of females in this treatment that exhibited corpus luteum in D17 was higher (90.0 vs. 30.0% - P<0.05).

Conclusions: The conclusion of this study was that the use of equine Chorionic Gonadotropin accelerates follicular development and improves the ovulation rate in Fixed-Time Artificial Insemination protocols in acyclic cows.

Reproduction

P02-002-183

Incidence of luteal phase inseminations and fertility rates in indigenous Nigerian Bunaji cowsHassan Mai^{1,*} Ambose A. Voh² Mohammed Sanusi¹¹Animal Production, Abubakar Tafawa Balewa University, Bauchi, Nigeria, Bauchi, ²Artificial Insemination Unit, National Animal Production Research Institute ABU, Shika-Zaria, Nigeria

Objectives: There is a paucity of information on the extent to which Nigerian indigenous cows are inseminated when they are not in oestrus, resulting in dwindling conception rates. The present study was undertaken to investigate the incidence of luteal phase inseminations and fertility rates in artificially inseminated Nigerian Bunaji cows.

Materials and Methods: A total of 269 normally cycling Bunaji cows aged between 4 and 9 years were used for this study. Double injection of 25mg Prostaglandin F_{2α} (PGF_{2α}) analogue (Lutalyse® Upjohn Co. MI) at 13 days interval was used to synchronize oestrus (Voh, Jr. et al. 2004). Visual oestrus detection and oestrus detection aids, namely tail-painting, KaMar heat mount detectors and vasectomized bull harnessed with a chin-ball mating device were all applied at the time of second PGF_{2α} injection (Mai et al., 2002). Both natural and induced oestruses were studied. Peripheral serum progesterone concentrations (SPC) at insemination and day 21 post-insemination were determined by a solid phase non-extraction I¹²⁵-Radio immunoassay method. The SPC at insemination was used to determine LPI. Cows with P₄ levels equal to or greater than 1.0ng/ml were considered to be in the luteal phase during AI hence the insemination considered to be a LPI (Coppens et al., 1987). Pregnancy was determined by SPC at day 21 after insemination and confirmed by rectal palpation 35 – 40 days post-insemination.

Results: The results revealed that out of the 560 inseminations carried out, the incidence of luteal phase inseminations (LPI) was 126 (22.5%). Variations in the post-service interval spanned between 4 and 57 days. The oestrus detection rate (70%), overall conception rate (32.0%), first service conception rate (51.6%) and number of services per conception (3.13) were considered unsatisfactory. Five cows conceived to the luteal phase insemination. The interval from one oestrus to another oestrus exhibited or incorrectly identified during the luteal phase (LPI) ranged from 27 to 39 days. Those cows inseminated in the luteal phase returned to oestrus between 4 and 15 days period. However, cows that failed to conceive in the same group, in spite of detection of oestrus and AI, manifested oestrus 18 to 57 days later.

Conclusions: Some of the factors likely to be responsible for the LPI include "Phantom heat" and management errors in heat detection on the part of the herdsmen, incorrect identification of animals, breakdown in communication between farm staff and inseminator or inadequately marked cows where heat detection aids were used. The results of the study have confirmed the incidence of LPI in Bunaji cows and this may contribute to the reduced conception rates observed in AI programmes in Nigeria.

Reproduction

P02-002-185

Investigation on mortality caused by calving disorders in cowsGiuseppe Martano^{1,*}¹Veterinary Service, ASL TO3, Vigone (TO), Italy

Objectives: the objective of this work was to evaluate the mortality prevalence due to calving disorders in bovine. there are several common problems that affect cows during calving: dystocia, laceration, rupture, uterine prolapses, retained placenta, which pose a great threat to production and reproduction efficiency of animals. In this study we considered two breeds : the Holstein and the Piemontese (an local italian breed).

Materials and Methods: We have studied a sample of 290 bovine herds located in northwest Italy. In these farms were raised 25800 bovine of different breeds. The length of this study was twelve months, from January to December and particularly they were examined calving data about 5629 holstein cows and 5410 Piemontese breeds cows. During the period under study were registered 4503 calvings in the Holstein cows and 4320 calvings in Piemontese breed cows. We collected data about age, breed, feeding, possibility of moving, management, staff experience.

Results: In this study we recorded 37 deaths caused by calving disorders in Holstein cows and 29 in Piemontese cows and we have examined data about age, breed, feeding and management. The mortality rate due by calving disorders was respectively 0,82 % in Holstein and 0,67 % in Piemontese cows. The risk of dystocia increased parity in Holstein and decreased in Piemontese breed. We have not found significant differences about season and feeding. We have found that a factor which was most decisive was the degree of readiness of farmer staff.

Conclusions: Risk factors for calving related disorders for dairy and breeding cows could be multiple and various. The relatively high mortality during calving found in this survey suggests a increased care in herd's management. The highest mortality rate was recorded in poorly managed herds.

Reproduction

P02-002-186

Use of Different Protocols Combining GnRH and Prostaglandin Analogues for the Treatment of Ovarian Cysts in Dairy CowsAna Cristina Figueiredo^{1,*} Carlos Fernandes^{1,2} Joao Henrique Viana³ Mauricio R. Vieira⁴ Denis A. Antonio⁵ Henderson Ayres⁵ Joao Paulo Barbuio⁵¹Animal Reproduction, Biotran LTDA, ²Animal Reproduction, University of Alfenas, Alfenas, ³Animal Reproduction, Embrapa - Dairy Cattle, Juiz de Fora, ⁴Animal Reproduction, Consulte Consultoria, Governador Valadares, ⁵MSD Saúde Animal, São Paulo, Brazil

Objectives: Ovarian cysts are a frequent pathological condition in dairy cattle Two types of ovarian cysts were described, follicular and luteinized. As the degree of luteinization of ovarian cysts is variable, adding prostaglandin F_{2α} to GnRH treatment may results in better

treatment outcomes than GnRH treatment alone. The aims of this study were to determine the incidence of follicular and luteal cysts in dairy herds in Southwest Brazil, and to evaluate the effectiveness of different treatment protocols using a GnRH combined or not with prostaglandin F2alpha.

Materials and Methods: Holstein cows (N=1,100) from four dairy farms located in the Minas Gerais State, Brazil, were evaluated in the post-partum period (days 28 to 40 days) by ultrasonography (Mindray M5, Mindray, SP, Brazil; linear 7.5MHz transducer) to detect ovarian cysts, characterized as anechoic structures 20 mm or greater in diameter. The cysts with a thin wall were classified as follicular, while those with the presence of a thick wall with the characteristic echotexture of luteal tissue were classified as luteinized. The cows previously diagnosed as bearing follicular or luteinized ovarian cysts were allocated into five groups, balanced according the type of cysts and days post-partum: G1 (Control Group; n = 16), 2 mL of saline; G2 (n = 31), 0.5 mg of Gonadorelin (GO) (Fertagyl™ MSD Saúde Animal, São Paulo, Brazil) ; G3 (n = 28), 0.5 mg of GO and 0.53mg Sodium cloprostenol (SC) (Ciosin™ MSD Saúde Animal, São Paulo, Brazil) 10 days later; G4 (n = 29) 0.5mg GO and 0.53mg SC given at the same moment; and G5 (n = 32), 0.5mg GO and two SC (0.53mg) shots, the first given at the same moment of GO and the second one 10 days later. All treatments were injected im. All treated cows were re-evaluated between 7 and 10 days after the end of each treatment. The continuous value parameters were assessed for normality, evaluated by ANOVA, and differences among treatments compared with Tukey's test (SAS Software, version 9.3). Differences in the efficiency of treatments were compared by the Chi-squared method. A p-value of 0.05 indicated statistical significance.

Results: Treatment was defined as effective if, at 7-10 days after treatment, the ovarian cyst had disappeared and a corpus luteum had developed. There was no farm effect (P>0.05). Ovarian cysts were detected in 12.4% (136/1100) of the cows, from which 23.5% (32/136) were classified as luteinized. The effectiveness of the treatments were differed between groups (18.75%^a, 54.8%^b, 53.5%^b, 79.3%^c, and 81.3%^c, for G1, G2, G3, G4, and G5, respectively; P<0.05). The best treatments were those who initially using PGF and GnRH (G4 and G5). The interval treatment to the first service (61.2±17.9^a, 44.5±16.4^b, 30.9±12.6^c, 26.19±14.3^c and 18.3±10.2^d days) and treatment to conception (71.9±21.9^a, 60.8±19.4^b, 48.3±16.9^c, 46.1±15.6^c, and 35.1±14.3^d days for G1, G2, G3, G4, and G5; respectively) differed between groups (P<0.05). However, there was no difference in conception rates among groups (P>0.05). The use of SC in the beginning of the protocol resulted in the higher effectiveness of treatment in groups G4 and G5, probably because of its effects in luteinized cysts. Considering cows with only luteinized cysts the efficiency was 33.3^a, 42.9^a, 50.0^a, 87.5^b and 87.5^b – P>0.05, for G1, G2, G3, G4 and G5, respectively. However, when a second SC was included in the protocol (G5) there was a further reduction in the interval from treatment to conception, when compared to G4. This reduction occurred probably because the second SC injection promoted the lysis of corpora lutea eventually formed after the earlier GO injection.

Conclusions: The incidence of ovarian cysts in the 4 study herds is 12.4%. Combined treatment of ovarian cysts with cloprostenol and gonadorelin is effective especially if it is followed by a second cloprostenol injection 10 days later.

Comments: Thanks: Fapemig, CNPq and CAPES

Reproduction

P02-002-187

Estimating of Weight from the Coronet Width of the Bovine Fetus before Delivery by Ultrasound Images

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Objectives: Dystocia is defined as delayed or difficult parturition. General causes are fetal-maternal size mismatch, fetal malpresentation and maternal-related causes such as uterine torsion. In particular, an excessive fetus causes a dangerous condition for both a cow and a calf. The size of the fetus has been estimated from the size of the hoof by rectal examination in clinical practice. However, this method was not effective due to lack of accuracy. The purpose of this study was to estimate the weight from the coronet width (hoof width) of the fetus just before delivery by ultrasound images.

Materials and Methods: A total of 78 calves, Jersey(male 12 female 6), Japanese Brown(male 12 female 8), Japanese Black(male 2 female 2) and Holstein (male 19 female 17), was used from 2013 to 2015. Experiment A: Measurement of the hoof crown width of fetal forelimb coronet width (hoof width) was measured using an ultrasonography (linear type 6-10MHz, Ezaote Inc.) per rectum from 10 days before the expected date of delivery. Gestation period of dairy and beef cattle were 280 and 285 days, respectively. Experiment B: The relationship between the hoof width and the body weight was measured by using an ultrasonography (the water immersion method: echo value) and a caliper (measured value) within 24 hours after delivery. Body weight of calves it was measured with a digital scale. (Minimum measured quantity: 50g). We have calculated the relationship between the hoof width (Echo value-measured value) and weight. It was applied to actual cases.

Results: Experiment A: The hoof width of the fetus was measured from the ultrasound image of the middle phalanx distal cross-section. Experiment B: Jersey, Holstein, Japanese brown cattle, Japanese black cattle of gestation days (mean ± SD) was 282.1 ± 4.5, 276.0 ± 5.4, 284.3 ± 3.8, 290.8 ± 2.6, each body weight (kg) was 28.4 ± 4.0, 40.0 ± 5.1, 34.6 ± 4.8, 27.3 ± 3.6, and each hoof width measured value (mm) was 53.9 ± 2.2, 57.4 ± 2.8, 56.7 ± 3.3, 52.0 ± 3.0, respectively. The relationship between the hoof width (Measured value) and hoof width (echo value) was Y = 0.9933X + 1.1124 (R² = 0.9574, P <0.001). The relationship between the measured value of the hoof width [X] and the weight [Y] is, Holstein: Y = 1.444X-42.907 (R² = 0.627, P <0.001), Japanese brown cattle: Y = 1.353X-42.110 (R² = 0.860, P <0.001). By measuring the hoof width of fetus using ultrasonography, it was found to be about the same as the measured value. If the fetal weight would be estimated before birth using this formula, it is possible to treat to avoid dystocia. Exactly using the weight estimation for excessive fetal of Japanese brown cattle, it was found that this way was useful method. We want to strive to applications in calving management practices by increasing the number of examples.

Conclusions: The hoof width of the fetus could be accurately measured by ultrasound imaging. Strong correlation was observed between the hoof width and weight of the calf. This study suggested that it is possible to estimate the weight of the fetus by measuring the hoof width of the fetus in the ultrasound image.

Reproduction

P02-002-188

The effect of feed on the postpartum ovarian dynamics and the body condition score of grazing breeding beef cattle

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Objectives: In previous studies, we reported that cows calving in January and February, compared with those calving in March and April, experienced delays in the recovery of their body condition score (BCS) and the start of their first ovulation after giving birth. These studies suggested the importance of energy intake before parturition in order to improve reproductive performance. The purpose of this study was to clarify if feeding supplementary concentrations to pregnant cows before calving would have an effect on their first ovulation and their body condition score (BCS) after parturition.

Materials and Methods: The Aso region is a highland (altitude 500 to 800m) located in the warm southwestern region of Japan but is relatively cool even in the summer. The average temperatures in February, March and April are 2.9 °C, 7.7 °C and 11.4 °C, respectively. The majority of Japanese beef cattle give birth seasonally, primarily in the spring, and breed in a pasture-based system in the Aso region. The study was conducted on 32 cattle (28 Japanese brown and 4 Japanese black) grazing on natural pastureland within Tokai University. Cattle were divided into the following 4 groups: W1, W2, S1 and S2 (W1: n=9, calving in 2012-2014. W2: n=5, calving in 2015. S1: n=14, calving in 2012-2014. S2: n=4, calving in 2015). W1 and W2 groups were delivered in January and February. S1 and S2 groups were delivered in March and April. Cows were kept in free grazing in grassland from April to October, eating about 10kg per head per day, and then in restricted feeding of 8kg per head per day of DMI grass silage from November to March. W2 and S2 were fed the supplementary concentrates from January to March. The ovaries and uterus were monitored by rectal palpation and ultrasonography from parturition to the next pregnancy; at 2 to 14 day intervals between January and August from 2012 to 2015. Evaluation of BCS using a 5-point scale with quarter-point divisions (Ferguson, 1994) was performed until confirmation of pregnancy.

Results: Feeding period (days, mean ± SD) of supplementary concentrates in W2 and S2 was 25.0±5.6 and 66.0±17.9, respectively. First ovulation days in postpartum in W1, S1, W2 and S2 was 67.4±16.1, 51.2±17.1, 63.0±17.3 and 34.3±11.5, respectively. The first ovulation of S2 was earlier than W1 (P<0.05). The BCS of S2 (3.25 ± 0.20) was higher than W1 (2.58 ± 0.35) and S1 (2.43 ± 0.36) at the time of delivery (P<0.05). It is thought that the lower BCS in the W2 group before and after parturition was due to a shorter feeding period of the supplementary concentrates. It is thought that ovulation could occur soon and the loss of BCS could be prevented in the W2 group if they are given the supplementary concentrates 2 months prior to delivery, as with the S2 group. The BCS of S2 (3.13 ± 0.14) was also higher than W1 (2.33 ± 0.28), S1 (2.46 ± 0.36), and W2 (2.40 ± 0.29) at the time of first ovulation (P<0.05), and was maintained the same level before and after parturition.

Conclusions: For preventing the loss of BCS, it was suggested that it would be effective to add supplementary concentrates from 2 months pre-birth. The present study indicated that it was important to prevent the loss of BCS before calving in reproductive management. In the future, it is necessary to examine the feeding amount and period of the supplementary concentrates.

Reproduction

P02-002-189

The Effects of Salpingitis on Bovine Reproductive Success - New Concepts As Seen by Live Cell Imaging

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Objectives: According to our studies, 30 -50 % of the cows slaughtered in European abattoirs reveal salpingitis. Inflammation of the oviduct is often associated with endometritis, but may also occur independently. As seen in our previous studies, salpingitis is a major cause for subfertility and idiopathic infertility. Therefore the aim of this study was to elucidate the effects of inflammation on sperm transport, sperm-oviduct interaction, oocyte transport, fertilization and early embryogenesis under near in vivo conditions.

Materials and Methods: For this purpose, oviducts revealing mild (n=35), moderate (n=30) and severe (n=22) inflammation were removed from cows immediately after slaughter. Oviducts with mild inflammation did not reveal macroscopic alterations, but showed increased vascularization in the stereomicroscopic investigation and an increased number of lymphocytes in the histomorphological analysis. Moderate inflammation was diagnosed by macroscopically visible increased vascularization whereas severe inflammation was associated with reddening of the tissue and visible amounts of secretion in the tubal lumen. Using a digital videomicroscopic live cell analysis system, which allows quantitative and qualitative assessment in real-time and under near in vivo conditions, the following parameters were investigated in the inflamed oviduct as compared to controls: a) mechanisms of transport of the oocyte and embryo, b) sperm-oviduct interaction and formation of the sperm reservoir, c) hyperactivation and detachment of sperm in the presence of the oocyte and d) fertilization and early embryogenesis. In addition to that, tissue samples with sperm and oocytes inside were analysed by stereomicroscopy, light microscopy, scanning electron microscopy and histochemistry.

Results: Our morphological investigations showed that inflammation in the oviduct was always correlated with increased numbers of lymphocytes being present in the epithelium and in the connective tissue. In addition to that, the capillaries in the mucosa were dilated and enlarged and increased secretory activity of the epithelium with accumulations of glycoproteins in the tubal lumen was seen. In severe inflammation, additionally increased apoptosis of the epithelial cells, loss of tight junctions, and shedding of epithelial cells into the lumen embedded in clumps of mucus were found. Live cell imaging revealed that particle transport speed (PTS) was significantly reduced resulting in retarded transport of the oocyte and the early embryo. Additionally ciliary beat frequency was decreased. In two individuals with moderate inflammation, cilia were immotile in specific regions of the epithelial folds. Accumulation of mucus resulted in sticking of the fertilized oocyte to the ampullar epithelium impairing timely transport of the early embryo in the oviduct. When huge accumulations of secretions were present sperm were stuck preventing them to form a sperm reservoir resulting in the loss of fertilizing capacity.

Conclusions: Our results imply that inflammation of the oviduct has a major negative impact on bovine reproductive success by compromising oocyte and embryo transport and by reducing survival time and fertilizing capacity of sperm. Thus, new diagnostic strategies such as the validation of a glycoprotein biomarker for salpingitis have to be developed. Furthermore new therapeutic concepts focusing on specific treatment of the oviduct, which aim to decrease secretory hyperactivity, would enable to further increase success rates of artificial insemination.

Reproduction

P02-002-190

Serum paraoxonase-1 activity and nonesterified fatty acid concentration in relation to the resumption of ovarian activity in postpartum dairy cows

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Objectives: The resumption of ovarian activity after calving is closely related to the severity of negative energy balance (NEB) and inflammatory response in the early lactation of dairy cows. Thus, the aim of the study was to evaluate nonesterified fatty acids (NEFA) concentration and paraoxonase-1 (PON1) activity related to the resumption of ovarian activity in postpartum dairy cows.

Materials and Methods: A total of 24 Holstein dairy cows were included in the study. The cows were assigned into two groups according to gynecological examination (rectal palpation and ultrasound examination) and progesterone (P₄) concentration: (1) ovulatory cows (n=11) which resumed ovarian activity within 60 days after calving; and (2) anovulatory cows (n=13) which did not resume ovarian activity during the same period. Blood samples were taken on days 5, 12, 19, 26 and 60 postpartum. Serum P₄ was measured by the immunoassay on Immulite 2000 analyzer (Siemens Healthcare Diagnostics, Germany); NEFA concentration was measured by Randox NEFA kit on biochemical analyzer SABA 18 (AMS, Rome, Italy) while high-density lipoprotein-cholesterol (HDL-C) concentration was assayed by the method based on selective inhibition of the non-HDL fractions by means of polyanions on Beckman Coulter AU 680 (Beckman Coulter Biomedical Ltd., Ireland). The PON1 activity was measured by the spectrophotometric assay using paraoxon as a substrate on Beckman Coulter AU 680. Statistical analysis was performed by SAS 9.3 Software. Results are shown as least squared means with 95% confidence interval.

Results: Serum NEFA concentration was significantly higher (P<0.05) in anovulatory cows postpartum (0.7 mmol/L) in comparison to the cows which resumed their ovarian activity within 60 days after calving (0.48 mmol/L). Anovulatory cows had significantly lower (P<0.05) PON1 activity after calving (228 U/L) than cows which resumed the ovarian activity postpartum (339 U/L). Accordingly, HDL-C concentration was significantly lower (P<0.05) in anovulatory cows after calving (1.8 mmol/L) compared to the cows which resumed the ovarian activity within 60 days postpartum (2.7 mmol/L).

Conclusions: Lower PON1 activity and higher NEFA concentration in anovulatory cows could indicate oxidative stress and inflammatory responses presenting a higher risk for decreased ovarian activity and thus lower reproductive efficiency of dairy cows after calving. Since serum PON1 is associated to HDL particle, lower HDL-C level in anovulatory cows might suggest the remodeling of HDL particle due to oxidative stress and inflammation. However, further studies are needed to elucidate the roles of PON1 and NEFA in decreased ovarian activity in postpartum dairy cows.

Reproduction

P02-002-191

Survey on impact and effect of Caesarian section on postpartum fertility in Piedmontese beef cattle

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Objectives: Piedmontese is a double muscle breed and then Cesarean section (C-section) is one of the most applied surgical techniques and is applied as routine field surgery, although complications during and after surgery are not unusual and should not be underestimated. This technique is believed to be associated with an increased risk of subsequent infertility and culling risk in cows. The objective of this study is to analyze indications and consequences of the C-section considering post surgery infections, therapy, resumption of ovarian cyclicity and partum to conception time.

Materials and Methods: Two skilled veterinary practitioners with homogeneous surgical protocols performed 174 C-sections on Piedmontese beef heifers and cattle, in 58 different farms, during a year of work. The data about farm, cause of the C-section, the surgical procedures, the subsequent pharmacological treatment were collected through within a few hours after surgery. As working procedure a check list of critical points was prepared in order to standardize the data collection. 154 cows were enrolled in the post surgical protocol and submitted to three or more postpartum clinical and ultrasound examination (1st visit 10-20 days/pp, 2nd visit 30-35 days/pp 3rd visit 90-100 days/pp, etc.) in order to collect all the information on post surgical complications and to evaluate the reproductive performances of cows.

Results: Collected data showed the incidence of different causes for surgery (calf macrosomia, 51.7%; dystocic parturition 16.1%; narrowness of the birth canal 10.9%). Partum season, geographical area and housing conditions have not affected post partum fertility (P>0.05). Neither suckling cows nor the presence of the calf twice a day show significant effect on fertility; all subjects showed resumption of ovarian cyclicity within 60 days postpartum (P<0.04). In accordance with the literature about beef cows these animals demonstrate partum to conception interval of 97 ± 30 days, compared with 157 ± 69 days of Friesian.

The differences in surgical protocol didn't affect the subsequent reproductive performances of cows, 62.3% subjects had a resumption ovarian cyclicity within 60 days postpartum, the 48.3% of cows conceive within 90 days postpartum and 82.8% within 120 days postpartum. The association of oxytetracycline hydrochloride systemic together with intrauterine administration greatly improves the reproductive performance in treated subjects, mainly in subjects with retained placenta, that show a resumption of ovarian cyclicity within 60 days post partum in a higher number of subjects (P = 0.03).

Conclusions: C-section has an average incidence of 10-15% within the Piedmontese cattle breed (heifers and cows) and is routinely applied on field with all the risks of an intervention carried out in non-sterile environment. Nevertheless a good practice, careful management and post surgery appropriate treatments are important to reduce detrimental effect on the reproductive career of submitted cows. Based on these considerations 88.5% of the cows undergoing cesarean section in this study have a chance to become pregnant within 120 days after surgery.

Reproduction

P02-002-192

Testicular hemodynamic changes in ram according to the season of the yearMalgorzata Domino¹·K Borzyszkowski²·Tomasz Jasinski³·Zdzislaw Gajewski¹¹Dep. of Animal Reprod, CBB, WCB, Warsaw University of Life Sciences, ²Dep. of Animal Reprod, CBB, WCB, Dep. of Animal reproduction, Warsaw University of Life Sciences, ³Dep. of Animal Reprod, CBB, WCB, FVM, WULS, Warsaw, Poland

Objectives: The numerous ram's reproductive parameters vary according to the season of the year. Nevertheless, the pattern of blood flow in the ram testis as well as the mean values for Doppler measures of blood flow in the testicular artery of the ram over the year were not yet to be estimated. The object of this study was to evaluate the testicular hemodynamic changes using pulsed wave Doppler sonography during and out of the breeding season.

Materials and Methods: Both testes from each of eighteen free of testicular pathology rams were examined using color and pulsed wave Doppler ultrasound with a linear array 3-13 MHz probe. Vessels of the epididymal edge of spermatic cord seen on two-dimensional ultrasound image were first detected with color Doppler and then blood flow of testicular artery was described with pulsed wave Doppler spectrum. Peak systolic velocity (PSV), end diastolic velocity (EDV), resistive index (RI), and pulsatility index (PI) of the testicular artery were measured in every examination both during and outside the breeding season.

Results: The parameters values (mean ± SEM) were PSV (cm/s): 28.45 ± 0.53, 33.78 ± 1.24; EDV (cm/s): 7.96 ± 0.26, 8.71 ± 0.42; RI: 0.71 ± 0.01, 0.71 ± 0.01; PI: 11.38 ± 0.37, 1.51 ± 0.49 during and outside the breeding season respectively. We found that: (1) all measures were obtainable; (2) measures for left and right testes were comparable ($P > 0.10$); (3) PSV ($P < 0.0001$) as well as PI ($P = 0.039$) were statistically significantly higher out of the breeding season compared to breeding season; (4) there were no significant differences ($P > 0.05$) between EDV ($P = 0.165$) as well as RI ($P = 0.520$) measures during and outside the breeding season.

Conclusions: According to the season of the year, the differences in ram's testicular size, sperm production, and mating capacity have been reported, within significance increase during the normal fall breeding season. On the other hand significance decrease of blood flow in the testicular artery (PSV and PI) during breeding season were observed. Resulting measures from all free of testicular pathology rams may provide useful reference values for clinical evaluation.

Reproduction

P02-002-193

Effect of shortening a synchronization protocol with a progesterone device (5d vs. 7d) for first AI in heifers on reproductive performance during the breeding seasonAlessio Valenza¹·E Hayes²·K Timms³·A H Souza⁴¹Ceva Animal Health, Libourne, France, ²Synergy Farm Health, Evershot, ³Ceva Animal Health Ltd, Amersham, United Kingdom, ⁴University of California Co-operative Extension, Tulare CA, United States

Objectives: The objective of this study was to compare two different synchronization protocols for fixed timed-AI with a progesterone releasing intravaginal device (PRID 5d vs. PRID 7d) applied at the beginning of the breeding season on reproductive performance in Holstein dairy heifers.

Materials and Methods: A total of 434 New Zealand Friesian heifers, kept in 4 pasture based seasonal calving UK herds were randomized to receive two synchronization protocols for 1st AI: 1) PRID5d: D0-8:00 am = progesterone releasing intravaginal device (PRID[®] Delta, Ceva Animal Health) plus 100 mcg of GnRH (gonadorelin diacetate) i.m. (Ovarelin[®]/Cystoreline[®], Ceva Animal Health); D5-8:00 am = PRID[®] Delta removal and treatment with 25mg of PGF2a (dinoprost) i.m. (Enzaprost[®]T, Ceva Animal Health); D6-8:00 am = treatment with a second dose of 25mg of PGF2a i.m.; D8-8:00 am = 100 mcg of GnRH i.m. (Ovarelin[®]/Cystoreline[®], Ceva Animal Health) plus AI; and 2) PRID7d: D0-8:00 am = PRID[®] Delta; D6-8:00 am = treatment with 25mg of PGF2a (Enzaprost[®]T) i.m. ; D7-8:00 am = PRID[®] Delta removal; D9-4:00 pm = AI. After this first timed AI, heifers were placed on the same pasture with cleanup Holstein bulls in the estimated ratio of 1 bull to 25 non-pregnant heifers. Pregnancy diagnosis was performed by ultrasound at intervals of 30 days throughout the breeding season that lasted 12 weeks. Statistical analysis was performed with proc Glimmix and proc Lifetest of SAS.

Results: Overall, heifers that received the short synchronization protocol (PRID5d) for first AI had an increased proportion of pregnancies at week 3 (PRID5d = 69.9% vs. PRID7d = 57.8%; $P = 0.01$), 6 (PRID5d = 91.4% vs. PRID7d = 81.1%; $P = 0.10$), or 12 (PRID5d = 100.0% vs. PRID7d = 99.8%; $P > 0.10$) compare to the ones that received the standard synchronization protocol (PRID7d). The survival analysis indicated that heifers enrolled on the short progesterone-based timed AI protocol (PRID5d) attained the 75% pregnancy mark two weeks sooner than the ones receiving the 7 days progesterone-based protocol (PRID7d) during the breeding season.

Conclusions: In conclusion, heifers enrolled in the shorter progesterone-based timed AI protocol (PRID[®] Delta, Ceva Animal Health) for first artificial insemination (PRID5d) conceived faster during breeding season, anticipating calving and allowing heifers to start milking sooner.

Reproduction

P02-002-194

GnRH-induced LH profiles following Gonadorelin diacetate vs. Buserelin treatment in lactating dairy cows.Alessio Valenza¹·R Armengol-Gelonch²·J M Mallo³·D Ponte²·A Jimenez⁴·A H Souza⁵¹Ceva Animal Health, Libourne, France, ²Lleidavet, Lleida, Spain, ³Lleidavet, Lleida, , ⁴Ceva Salud Animal, A, , ⁵University of California Cooperative Extension, Tulare CA, United States

Objectives: The study was designed to characterize the GnRH-induced LH surge profile under high (Diestrus) or low (Pro-estrus) circulating progesterone in lactating dairy cows after treatment with two different types of GnRH products commonly used in breeding procedures of lactating dairy cows. The secondary objective was to investigate whether season (summer vs. winter) could alter LH surge profile.

Materials and Methods: Lactating Holstein cows at 108.2±2.3 days in milk (DIM), producing 41.5±0.3 kg/day were randomized to receive, during proestrus and diestrus (7 days after ovulation): 1)Cystoreline/Ovarelin[®] i.m. (n = 56; 2mL, 100 mg of Gonadorelin diacetate tetrahydrate;

Ceva Animal Health) or 2) Receptal® (n = 52; 2,5mL, 10 mcg of Buserelin diacetate; MSD). Data collection was done in two replicates (summer vs. winter) using different cows. Blood samples were collected at hour 0 (just before GnRH treatment), at 30min, 1h and then hourly until 5h post-GnRH treatment. Only cows having complete luteolysis (P4 1ng/mL) during diestrus were included in the analysis.

Results: Overall, There were no interactions between GnRH type and phase of the estrous cycle or season. Thus, peak LH concentrations (ng/mL) were not affected by type of GnRH (Cystoreline/Ovarelin® = 6.2±0.4 vs. Receptal® = 6.7±0.4; P=0.37) or season (winter = 6.8±0.4 vs. summer = 6.1±0.4; P=0.22), but were largely affected by phase of the cycle (proestrus = 8.2±0.4 vs. diestrus = 4.7±0.4; P

Conclusions: In conclusion, cows receiving Gonadorelin diacetate had greater circulating LH concentrations (ng/mL) at 1h after GnRH treatment than cows receiving Buserelin (4.2±0.3 vs. 3.1±0.3; P

Reproduction

P02-002-195

Effect of decreasing the duration of a resynchronization protocol with a progesterone device and addition of a second PGF2α treatment on fertility in lactating cows

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Objectives: The objective of the study was to evaluate the effects of (1) decreasing the interval between the first GnRH (G1) and the PGF2 (PGF) treatment from 7 to 5 days, (2) adding a second PGF treatment 24 h after the first and (3) the degree of vaginal discharge at the time of the progesterone releasing intravaginal device (PRID® Delta, CEVA Animal Health) removal on P/AI during a resynchronization protocol for fixed timed artificial insemination (TAI) in lactating dairy cows.

Materials and Methods: Lactating Holstein cows (n = 821) were randomly assigned at a non pregnancy diagnosis (d 0) to one of three resynchronization protocols: 1) 7D1PGF: D0 = progesterone releasing intravaginal device (PRID® Delta) plus 100 mcg of GnRH (gonadorelin diacetate) i.m. (Ovarelin®/Cystoreline®, Ceva Animal Health); D7 = PRID® Delta removal and treatment with 25mg of PGF2 α (dinoprost) i.m. (Enzaprost®T, Ceva Animal Health); D9.5 = 100 mcg of GnRH i.m. (Ovarelin®/Cystoreline®); 2) 7D2PGF: D0 = PRID® Delta plus 100 mcg of GnRH (gonadorelin diacetate) i.m.; D=7 PRID® Delta removal and treatment with 25mg of PGF2α (dinoprost) i.m.; D8 = second PGF2α (dinoprost) treatment ; D9.5 = 100 mcg of GnRH (gonadorelin diacetate) i.m.; and 3) 5D2PGF: D2 = PRID® Delta plus 100 mcg of GnRH (gonadorelin diacetate); D=7 PRID® Delta removal and treatment with 25mg of PGF2α (dinoprost); D8 = second PGF2α (dinoprost) treatment ; D9.5 = 100 mcg of GnRH (gonaderlin diacetate) i.m. All cows received timed-AI approximately 16 h after the second GnRH treatment. The degree of vaginal discharge was evaluated at the time of PRID® Delta removal and blood samples were collected at the time of the last GnRH treatment and evaluated for progesterone concentrations. Pregnancy diagnosis was performed 32 ± 3 d and 60 ± 3 d after AI using a portable ultrasound scanner and statistical analysis was performed by logistic regression using the GLIMMIX procedure of SAS.

Results: Overall, cows receiving a second PGF2α treatment (7D2PGF + 5D2PGF) had more pregnancies per AI (P/AI) (42.6% vs. 35.7%) than cows receiving a single PGF2α treatment (7D1PGF). For cows treated with a second PGF2α treatment, decreasing the duration of the progesterone-based protocol did not increase P/AI (41.4% vs. 43.8% for 7D2PGF vs. 5D2PGF). In addition, neither the number of PGF2α treatments (8.5% vs. 10.6%, for 7D1PGF vs. 7D2PGF + 5D2PGF) nor the duration of the protocol (9.1% vs. 12.1%, for 7D2PGF vs. 5D2PGF) affected pregnancy loss from 32 to 60 d after TAI. The percentage of cows with incomplete luteal regression at the second GnRH treatment tended to be different among treatments and was least for 7D2PGF cows, intermediate for 5D2PGF cows and greatest for 7D1PGF cows (1.9% vs. 6.9% vs. 11.0%, respectively).

Conclusions: In conclusion, the addition of a second PGF treatment to a progesterone-based protocol increased the percentage of cows with complete luteal regression and P/AI (7D1PGF vs. 7D2PGF) and decreasing the interval between G1 and the PGF treatment from 7 to 5 days did not improve P/AI. The degree of vaginal discharge at the time of removal of the progesterone releasing intravaginal device (PRID® Delta, CEVA Santé Animale) did not affect P/AI.

Reproduction

P02-002-196

Veterinary survey of the impacts of Mycotoxins in Cattle Food Production Systems

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Objectives: This survey based review carried out as part of a collaborative AHDB supported project aimed to assess the impact of mycotoxin exposure on the livestock industry through collating information on the experiences of vets and farmers with mycotoxins. They were asked to comment on different clinical presentations seen, the uptake and benefits of available diagnostic services and methods used to mitigate mycotoxin exposure. They were also asked to evaluate their perceived significance of mycotoxins, their knowledge, the availability of scientific evidence and the routes of knowledge transfer within the industry.

Materials and Methods: The surveys were designed specifically for veterinary and farmer populations and included open and closed questions to provide consistency in results. Vets at Bishopton Veterinary Group, Synergy Farm Health and The University of Nottingham trialled the surveys to ensure an appropriate time frame was required to complete the survey and they were written in an appropriate manner. Paper and electronic copies of the surveys distributed through BCVA, PVS, SVS, BPEX and the XLVets network as well as directly to farmers from Bishopton Veterinary Group, North Yorkshire and Synergy Farm Health, Dorset. This aimed to improve compliance and increase responses by providing the most appropriate response form for different groups. It also tried to ensure that an appropriate geographical distribution was obtained to be representative and minimise bias. Surveys were however restricted geographically to the UK to comply with the project budget.

The surveys were split into sections to focus on;

Case experience and management. Including; clinical presentations, uptake and experience if diagnostic services, individual toxin identification

Mitigating practices. Including; rationale behind binder inclusion, time periods and duration of binder inclusion, observed responses and evidence to support binder inclusion, other recommended mitigating methods

Knowledge transfer. Including opinions on; significance of the impact of mycotoxins, availability of scientific evidence, level of knowledge, knowledge transfer streams.

Survey results were collected & analysed in Microsoft Excel.

Results: Responses demonstrated that vets had greater experience with mycotoxin exposure at both individual & herd level than farmers. Use of testing services was limited among vets (32%) & farmers (18%). Respondents reported sending forage/feed samples, blood, bile, tissue samples, gut contents & water. Aflatoxins were most commonly tested for in cattle (22%), but a limited number of respondents (17%) reported obtaining positive results.

A large proportion of vets clients (72%) & farmers (49%) were including mycotoxin binders, the majority (85%) in dairy herds. The most common rationale for inclusion was risk based, during the winter period/housing 17% or based on clinical presentation (15%). The most commonly reported method used to monitor binder inclusion response was the resolution of clinical signs. A large proportion of respondents (40%) reported seeing no response following inclusion & the most common rationale (60%) for this was that mycotoxins weren't the underlying problem. Hygiene of feed storage and ongoing monitoring of environmental conditions to limit the development of mycotoxins were the most commonly recommended mitigating practices.

The majority of vets & farmers were in agreement that they were unsure of the significance of the impact of mycotoxins on livestock production. Their opinions differed in the availability of scientific evidence. Vets perceived feed companies as the main route of knowledge transfer to farms, whereas farmers saw vets as the source. Overall farmers rated their knowledge of mycotoxins as average, vets rated their knowledge as poor.

Conclusions: The clinical signs observed by vets and farmers were similar but consistently vague and non-specific. The inclusion of mitigating products appears to be commonplace but it is often with little clinical or diagnostic evidence, and with limited positive responses. The results confirmed this, demonstrating limited uptake of testing services from both vets and farmers. The perceived lack of evidence available to vets, combined with farmer reliance on this knowledge transfer route highlights areas where more industry focus is required to encourage consistent knowledge transfer.

Reproduction

P02-002-197

The Efficacy of a Single Dose of Equine Chorionic Gonadotropin for Superovulation of Cows

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Objectives: The Equine Chorionic Gonadotropin (eCG) is extracted from pregnant mares' serum. It is usually produced by the placenta of this species between 40 and 130 days of gestation. In bovines, it

can link to both FSH and LH receptors in the ovarian follicles. The main advantage of eCG is the induction of superovulation. Due to its extended half-life, we hypothesized eCG may need only one injection, without affecting efficacy, where other products need several applications to be effective. The objective was to verify the efficacy of one single dose of eCG (Folligon® MSD Animal Health - Brazil) for induction of super-ovulation of bovines.

Materials and Methods: Twenty cyclic crossbred females were used (10 cows and 10 heifers) randomly divided in crossover design. All females were submitted to two subsequent treatments that were repeated twice within a 40-day interval. The following protocol was used: D0: insertion of a progesterone implant plus application of 2mg estradiol benzoate; D4: 2,000IU (IM) of eCG (T1) or 10ml (IM) saline (T2); D8: removal of the implant and IM application of 0.5mg sodium cloprostenol (Ciosin® MSD Animal Health - Brazil); D9: IM application 0.1mg of Gonadorelin (Fertagyl® MSD Animal Health - Brazil). A Mindray M5 ultrasound 7.5 MHz linear transducer was used to evaluate the ovaries. Evaluations were carried out on D0 and from D4 to D9 on a daily basis. The number of ≥ 5 mm \emptyset follicles were counted during these evaluations. Another appraisal took place on D18 to determine the number of ovulations as per the number of corpora lutea. The animals were uniformly divided according to the number of ≥ 5 mm \emptyset follicles on D4 - prior to treatments of the first repetition. The data were submitted to a normality test and the mean number of ≥ 5 mm diameter follicles on each day of the evaluation was compared on D0 and from D4 to D9 using the Tukey's Test. On D17 the number of ovulations was also compared, using the Fisher's Exact Test. The significance level was considered as less than 5% probability and all statistical analysis were performed using the SAS program version 9.3.

Results: Cows and heifers data were analysed together (no category effect). On the randomization day, average number of follicles capable of responding to development stimulation (≥ 5 mm \emptyset) were unchanged (1.9 ± 0.8 and 2.1 ± 0.7 for T1 and T2, respectively - $P > 0.05$), showing females were uniformly distributed between treatments. No differences were observed of variables under control while comparing both repetitions ($P > 0.05$). The evolution of the number of ≥ 5 mm \emptyset follicles - considering both repetitions together - was as early as 24 h after application of eCG (D5), the number of follicles in T1 was $>$ T2 ($P < 0.05$) - and was maintained until D9. The average number of ≥ 5 mm \emptyset follicles on different days for T1 and T2, was respectively: D5: 10.0 ± 5.2 vs. 4.6 ± 1.7 ; D6: 14.5 ± 6.6 vs. 2.3 ± 1.9 ; D7: 19.3 ± 7.1 vs. 2.3 ± 1.6 ; D8: 24.1 ± 8.9 vs. 2.8 ± 2.0 ; D9: 24.0 ± 8.3 vs. 2.3 ± 1.7 . In addition, a gradual evolution of the average number of ≤ 5 mm \emptyset follicles between D4-D8 of animals from T1 was seen, showing eCG is capable of stimulating new follicles development for a long period after application. In the control group, there was a reduction of the average count of follicles during certain periods. Control animals were in follicular dominance and had atresia in part of the existing population. As a result of the eCG stimulation at the beginning of the follicular wave, more follicles reached the protocol end (D9) presenting hormonal conditions to ovulate, i.e., with a \emptyset of ≥ 9 mm (16.7 ± 4.8 vs. 1.2 ± 0.6 - $P < 0.05$). The ovulation rate from T1 was higher (16.0 ± 5.8 vs. 0.9 ± 0.5 - $P < 0.05$) than the control group (T2).

Conclusions: The conclusion is that the product Folligon® - while using the application schema of one single dose of 2000 IU of eCG is effective in promoting greater follicular development and super-ovulation of cows.

Reproduction

P02-002-198

The effect of natural and induced calving of beef heifers on maternal health and colostrum quality

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Objectives: The aim of this study was to determine the effect of natural and induced calving in continental cross beef heifers carrying pedigree Simmental calves on the subsequent cows' immune system, colostrum quality and subsequent reproductive health.

Materials and Methods: At day 285 of gestation, 55 crossbred recipient beef heifers carrying purebred Simmental embryos, were blocked by live-weight BCS, expected calving date and foetal gender and assigned to one of three groups: (i) control (no induction treatment; CON, n=19); (ii) induction with corticosteroids (CORT, n=20) and (iii) induction with corticosteroids plus prostaglandin (CORT+PG, n=16). Interval from induction to calving in hours and calving ease were recorded. Vaginal mucus samples were collected on day 21 (D21) and 42 (D42) post calving (D0) by means of a Metrichick® and scored on a scale of 0-3. Reproductive examinations were conducted on D21 and D42 days post calving and uterine cytology samples were obtained on D21. Blood samples were taken at 2 weeks before parturition (D-14), 1 day after parturition (D1) and 2 weeks after parturition (D14) for subsequent analysis of cortisol and calcium (Ca) concentrations. Calves were weighed at birth and subsequently every week until 10 weeks of age and a colostrum sample was taken immediately after the calving and stored for subsequent analysis. Data were analysed using Spearman correlation and stepwise linear regression using SPSS for Windows.

Results: There was a strong correlation between Ca concentrations on D14 and mucus score and cytology score on D21. Cortisol concentrations were not different between groups on D-14 but on D1 the control group had significantly higher cortisol concentrations compared with both treatment groups. On D14 the CORT+PG group had higher concentrations of cortisol when compared with the control group but these concentrations were not different than those of the CORT only group. IgG concentrations at calving did not differ between the two induced groups but were significantly lower in the control group ($P < 0.05$) when compared with the induced. Treatment group and Ca concentrations on D-14 were strong predictors of IgG concentration. Calf weight at birth was not different between groups and neither was the total weight gain over 10 weeks. There was a strong correlation between total weight gain and the weight gain per week. Calf weight at birth was correlated to mucus score, uterine involution and cytology on D21.

Conclusions: Induction of calving has no negative effects on calf weight gain but can have a positive effect on IgG concentrations in the colostrum. Calcium concentrations on D1 and D14 post calving do have an effect on subsequent reproductive health. Induction with cortisol leads to a decrease in cortisol concentrations at D1 post calving but this is a temporal effect.

Comments: This project was funded by the UCD Seed Funding Scheme - SF827

Reproduction

P02-002-199

Estimating the body weight of cattle fetuses before birth by measuring the coronet width with ultrasonography

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Objectives: Dystocia is defined as delayed or difficult parturition. The general causes are fetal-maternal size mismatch, fetal malpresentation, and maternal-related causes such as uterine torsion. In particular, an excessively large fetus can cause a dangerous condition for both the cow and the calf in clinical practice. Fetus size is usually estimated from the size of the hoof by rectal examination. However, this method is not effective due to lack of accuracy. The purpose of this study was to use ultrasound images to estimate the weight of a fetus just before delivery from the coronet width (hoof width).

Materials and Methods: A total of 78 calves, – Jersey (12 male, 6 female), Holstein (19 male, 17 female), Japanese Brown (12 male, 8 female) and Japanese Black (2 male, 2 female) – were studied from 2013 to 2015. Experiment A (measuring the hoof width of a fetus): The forelimb hoof width was measured using ultrasonography (linear type 6-10MHz, Ezaote Inc.) via rectum from 10 days before the expected date of delivery. Experiment B (the relationship between hoof width and body weight of the calf): The hoof width of a calf was measured using ultrasonography with the water immersion method (echo value) and a caliper (measured value) within 24 hours after delivery. The body weight of a calf was measured with a digital scale. (Minimum measured quantity: 50g). The relationship of the hoof width and weight was calculated and applied to actual cases.

Results: Experiment A: The hoof width of the fetus was measured from the ultrasound image of the middle phalanx distal cross-section. Experiment B: The gestation period (mean \pm SD) of the Jersey, Holstein, Japanese brown cattle and Japanese black cattle was $282.1 \pm 4.5, 276.0 \pm 5.4, 284.3 \pm 3.8, 290.8 \pm 2.6$, respectively; their body weight (kg) was $28.4 \pm 4.0, 40.0 \pm 5.1, 34.6 \pm 4.8, 27.3 \pm 3.6$; and their hoof width measured value (mm) was $53.9 \pm 2.2, 57.4 \pm 2.8, 56.7 \pm 3.3, 52.0 \pm 3.0$. The relationship between the echo value [X] and the measured value [Y] of the hoof width was $Y = 0.9933X + 1.1124$ ($R^2 = 0.9574$, $P < 0.001$). The relationship between the measured value of the hoof width [X] and weight [Y] is $Y = 1.444X - 42.907$ (Holstein, $R^2 = 0.627$, $P < 0.001$) and $Y = 1.353X - 42.110$ (Japanese brown cattle, $R^2 = 0.860$, $P < 0.001$). Using this equation to estimate fetal weight before parturition allowed for treatments to avoid dystocia, such as parturition induction. Using this weight estimation method was also effective in identifying excessively large fetuses in Japanese brown cattle.

Conclusions: The hoof width of the fetus could be accurately measured using ultrasound imaging. Strong correlation was observed between the hoof width and weight of the calf. This study suggested that it is possible to estimate the weight of a fetus by measuring the hoof width of the fetus using ultrasound image.

Reproduction: Beef

P04-004-184

Comparison of an alternative diagnostic sampling technique for *Tritrichomonas foetus* in cattle

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Objectives: Bovine trichomoniasis is emerging as a major concern in the beef industry. Recent advancements in PCR diagnostics have increased the ability to detect the disease in asymptomatic bulls. However, the greatest limitation is proper collection of an adequate sample. Furthermore, the low repeatability of most sample collection techniques can cause confusion and misdiagnosis. The aim of the study was to validate a sample collection technique that increased sensitivity and was easier and safer to collect than preputial scraping.

Materials and Methods: One hundred eleven commercial bulls of unknown infection status, were sampled for detection of *Tritrichomonas foetus* using two different collection methods: 1) traditional preputial scraping with a dry insemination pipette (TPS) and 2) penile preputial swabbing (PPS). TPS samples were taken by vigorously scraping preputial/penile mucosa using a rigid insemination pipette while applying negative pressure. PPS samples were obtained by briskly swabbing the penile and preputial mucosa with a gauze sponge during full extension of the penis. All samples were processed using InPouch™ TF media and submitted under similar conditions for PCR testing at ISU Veterinary Diagnostic Laboratory.

Results: Positive PCR results were observed in 37 out of 111 (33%) bulls using the traditional preputial scraping technique, however 39 out of 111 (35%) were positive using penile preputial swabbing technique. Sensitivity and 95% Confidence Intervals (CI) was determined with web based application utilizing R software. The Newton-Raphson algorithm predicted the sensitivity of the traditional preputial scraping method was 0.919 (CI 0.689-0.983) and the sensitivity of the penile preputial swabbing was 0.949 (CI 0.818-0.987).

Conclusions: This data indicates that the penile preputial swabbing technique is a reliable alternative to the traditional preputial scraping method. Regulatory veterinarians should consider allowing sponge samples as an official test for trichomoniasis.

Reproduction: Beef

P04-004-185

Effect of weaning, non-pregnancy and calf loss on live weight production and efficiency of beef cows in northern Australia

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Objectives: To measure annual live weight production and live weight production ratio of cows as a function of mating outcome which is either failure to conceive (E), calf loss (L) or wean a calf (W) during one year

Materials and Methods: Income from beef herds is primarily determined by live weight production. For a cow over one year this is measured

as change in her live weight (at weaning times) plus the weight of calf weaned. Live weight production for a breeding herd is a function of weaner numbers and weight, and cow growth and survival.

Brahman and Tropical Composite females (n=2,127) were first mated at 2 years of age and monitored between 2.5 and 8.5 years of age during which time they had the potential to wean six calves (Johnston et al. 2014). The four age groups of cattle used were allocated to graze pastures on the four major country types in Queensland where they were mated for three months annually. Live weight production ratio was calculated from the 11,566 mating outcomes as annual live weight production divided by the average live weight of the cow-calf unit over the year, the latter being an indicator of feed intake.

Results: Average annual live weight production was:

- 36 and 23 kg less on northern forest (150±43kg) and downs country (163±77kg), respectively, compared to central and southern Queensland (186±69kg).
- 29 kg less in the year after first mating, than subsequently (152±60kg v 181±72kg).
- 74 and 128 kg more in W cows compared to E and L cows (199±59kg, 125±42kg, 71±50kg, respectively). E cows started the year in lower body condition (2.6±0.8; 5-point scale) than L and W cows (3.2±0.7).
- 38-40 kg more if previous mating outcome was W (190±71kg) compared to L (152±75kg) or E (150±62kg).
- 222±53kg and 132±63kg in cows that raised six and three calves in six years, respectively. Cows weaning six calves were in better body condition at weaning (2.8±0.5 v 2.5±0.6) and weaned heavier calves (208±37kg v 201±36kg) than low-fertility cows. Cumulative live weight production was linear in both cow classes.

Average live weight production ratio was:

- 0.34±0.10 kg/kg of cattle for W cows compared to 0.26±0.09 and 0.14±0.10 kg/kg of cattle for E and L cows, respectively.

Conclusions: Calf loss has a larger impact on live weight production of breeding cows than non-pregnancy does, partly because non-pregnant cows are in lower initial body condition. Cows that wean a calf every year appear to have much higher inherent ability to produce live weight from available pasture than low-fertility phenotypes, as indicated by their higher live weight production, heavier weaners, better body condition at weaning and higher live weight production ratio. Variation in live weight production may be associated with the high genetic variation for ability to conceive in tropically-adapted cattle previously reported from this study.

Comments: Fordyce G, McGowan MR, McCosker K and Smith D (2014) Live weight production in extensively-managed beef breeding herds. In: Proceedings of the 28th World Buiatrics Congress, Keynote lectures, 27 July to 01 August 2014, Cairns. pp.87-93.

Johnston DJ, Barwick SA, Fordyce G, Holroyd RG, Williams PJ, Corbet NJ and Grant T (2014) Genetics of early and lifetime annual reproductive performance in cows of two tropical beef genotypes in northern Australia. *Animal Production Science*, 54:1-15.

Reproduction: Beef

P04-004-186

A Comparison of Double Prostaglandin (PG) and 5 Day Progesterone Implant Co-synch to Breed Beef Heifers with Timed Artificial Insemination (TAI)

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Objectives: A large (1300 cow) extensively managed commercial beef herd in South-West Scotland had been using AI for several years to breed large groups of heifers with variable success. The standard protocol used by the farm was double PG injection followed by TAI at 72 and 96 hours. In an attempt to improve previous TAI results and also reduce handling and AI costs a group of 110 heifers were synchronised with a 5 day CIDR co-synch protocol to compare to the herd's standard double PG breeding protocol.

Materials and Methods: Heifers were Aberdeen Angus, Simmental or Saler crosses and around 18 months of age at breeding in November 2014. The heifers were outdoors at grass and supplemented with silage and rationed cereal based concentrate (2kg/day) during the breeding period. A total of 276 heifers were mated with Double PG : day 1 and day 11 PG injection (0.15mg d-cloprostenol, Prellim@injection, Zoetis) followed by TAI 72 and 96 hours after second PG injection. 110 heifers were mated with 5 day CIDR co-synch protocol: day 1 CIDR (CIDR@1.38g, Zoetis) inserted plus GnRH injection (100µg gonadorelin, Acegon@, Zoetis). Day 5 CIDR insert removed and PG injection. Day 8 (72 hours later) TAI and GnRH injection.

All inseminations were carried out by trained AI technicians and pregnancy was confirmed at approximately 8-9 weeks post AI by ultrasound examination. A basic economic costing for breeding 100 heifers was modelled using manufacturer list price drug costs (CIDR £9.74, PG £2.76, GnRH £3.28) and AI cost of £12 per service (technician labour and semen). Vet and farm labour costs were not included. An average daily liveweight gain (ADG) of 1kg was assumed for calves and liveweight sale value of £2.20/kg. Conception rates were compared using a Chi-square test.

Results: Conception rate to Double PG protocol was 48% (133/276) and to CIDR Co-synch was 63% (69/110; P=0.01).

Economics modelled on 100 heifers bred to each protocol. Double PG protocol : drugs £552; AI costs £2400; cost per heifer bred £29.52; cost per pregnancy generated £61.50. CIDR Co-synch protocol : drugs £1906; AI costs £1200; cost per heifer bred £31.06; cost per pregnancy generated £49.30.

Potential Value of Superior 1st Serve Conception Rate. The superior conception rate to 1st service with CIDR co-synch delivered an additional value of 15 calves/100 heifers born in 1st cycle of mating which could generate an additional 15 x 21kg weaning weight advantage over calves born to next cycle = 315kg @ £2.20/kg LW = £693 (Assuming 1kg ADG and at least 21 days older at weaning).

Conclusions:

- On this farm during the same breeding season the CIDR co-synch protocol delivered superior results to the standard double PG protocol (63% vs 48% CR, P =0.01)
- The superior CR and reduced AI costs led to an economic saving of £12.20 per pregnancy generated despite the greater drug costs associated with CIDR co-synch
- A 15% difference in 1st service CR could also have been worth around £693 / 100 heifers bred due to extra kg of weaned calf weight or £46.20 per extra pregnancy to 1st service
- The 5 day CIDR co-synch protocol may offer significant economic and labour saving advantages over traditional double PG protocol for TAI in beef heifers.

Reproduction: Beef

P04-004-187

Effects of Injectable Trace-Mineral Supplementation on Reproductive Performance of Beef Cows and Developing Beef Bulls

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Objectives: Our objective was to evaluate the effects of bolus injections of a trace-mineral solution (TM; 15 mg/mL Cu, 10 mg/mL Mn, 5 mg/mL Se, and 60 mg/mL Zn) or an equal volume of physiological saline (SA) on pregnancy to timed insemination in beef cows (study 1) and on semen-quality characteristics in young beef bulls (study 2).

Materials and Methods: In study 1, beef cows (n = 460; initial BW = 497 ± 89 kg) were stratified by BCS, age, and predicted calving date and assigned randomly to either TM or SA. Treatments were administered to cows (1 mL/90 kg BW) 105 d before calving began and again 30 d before fixed-time AI. Following calving, cows with suckling calves grazed 1 of 5 native-range pastures; treatments were evenly distributed within pastures. Trace-mineral supplements and white salt were available to cows ad libitum before and during study 1. Blood samples were collected from cows 17 and 8 d before fixed-time AI. Serum progesterone was quantified by RIA. Ovulation was synchronized using a 5-d CO-Synch + CIDR protocol; cows were inseminated 72 h after CIDR removal. Cows were exposed to fertile bulls for natural-service breeding 10 d after AI for 40 d. Conception to AI and final pregnancy were assessed 36 d after AI via transrectal ultrasonography and 120 d after AI via rectal palpation, respectively.

In study 2, bull calves aged 7 months (n = 491; initial BW = 314 ± 45 kg) were stratified by breed and ranch of origin and assigned randomly to the same treatments used in study 1. Treatments were administered to beef bulls at 7 months of age (1 mL/45 kg BW) and at 10 months of age (1 mL/68 kg BW). Within treatment, breed, and source strata, bulls were assigned randomly to 1 of 8 pens, where they were fed a growing diet ad libitum for 225 d. The diet was formulated to promote a 1.5 kg ADG at a DMI of 2.6% of bull BW. Breeding soundness examinations (BSE) were conducted at 10 and 12 months of bull age.

Results: In study 1, change in BW and BCS from study initiation to calving and from AI to weaning did not differ (P≥0.15) between TM and SA cows. Conversely, TM cows had greater (P=0.04) BCS increase than SA cows between calving and AI. Proportion of cows with estrous cycles (serum progesterone ≥ 1 ng/mL) ≥ 8 d before fixed-time AI did not differ (P≥0.51) between treatments. In contrast, conception to fixed-time AI was greater (P=0.05) for cows receiving TM (60.2%) than for cows receiving SA (51.2%). Final pregnancy rate did not differ (P=0.24) between treatments and averaged 92%; however, a greater (P=0.01) proportion of TM-treated cows calved in the first 20 d of the subsequent calving season than SA-treated cows (77.5 vs. 65.0%).

In study 2, BW, ADG, and scrotal circumference of bulls were not affected (P≥0.16) by treatment. Proportions of TM- and SA-treated bulls receiving satisfactory BSE scores did not differ (P=0.98) at 10 months of age (50 % for both TM and SA). At 12 months of age, the proportion of TM-treated bulls receiving satisfactory BSE scores was numerically larger (P=0.43) than that of SA-treated bulls (89% for TM and 86% for SA). This change was associated with greater (P=0.05) sperm motility in TM-treated bulls than in SA-treated bulls at 12 months of age; moreover, TM-treated bulls had greater (P≤0.05) improvement in sperm morphology and sperm motility between 10 and 12 months of age than SA-treated bulls. Among bulls that failed BSE at 10 months of age, more (P=0.10)

TM-treated bulls passed BSE at 12 months of age than SA-treated bulls (98 vs. 94%, respectively).

Conclusions: Under the conditions of these studies, pre- and postpartum trace-mineral injections improved conception to fixed-time AI and subsequent calving distribution of beef cows. Sperm motility and morphology at 12 months of age were improved in bulls treated with injectable trace minerals at 7 and 10 months of age compared to bulls treated with saline. Among bulls that were sub-fertile at 10 months of age, more TM-treated bulls passed BSE at 12 months of age than SA-treated bulls

Reproduction: Beef

P04-004-188

A Double Synchronisation and Artificial Insemination (AI) Breeding Protocol for Beef Cows

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Objectives: The use of AI in beef cows at pasture is limited by the practical problems of heat detection and multiple handling. A double synchronisation protocol was developed to breed beef cows using AI with minimal heat detection and maximise the percentage pregnant to AI in the first 24 days of the breeding period.

Materials and Methods: Spring calving (April-May) Angus or Limousin crossbred beef cows on a single farm in Midlothian, Scotland were bred by AI as described below over four consecutive years. During the breeding programme described all animals were at pasture and suckling calves. Day 0 was defined as the start of AI breeding period. At day -11 cows had progesterone (P) implants (CIDR® 1.38g, Zoetis) inserted and received GnRH injection (0.01mg Buserelin, Receptal™, MSD Animal Health). Seven days later (day-4) all cows received prostaglandin (PG) injection (500µg cloprostenol, Estrumate™, MSD Animal Health). Two days later (day -2) CIDR implants were removed and any cow calved <55 days at this time also received 400iu serum gonadotrophin (PMSG-Intervet, MSD Animal Health) by i/m injection. Fixed time AI (TAI) was carried out twice at 48 and 72 hours after CIDR removal (days 0 and 1). After removal the CIDR implants were washed in dilute chlorhexidine, dried and stored for re-use. On day 16 all cows had the once-used CIDR implants inserted and left in place for 5 days. On day 21 the CIDR implants were removed, tail paint applied and cows observed at pasture for returns to oestrus over the next 72 hours. Cows were gathered on days 23 and 24 and inseminated based on observation of standing to be mounted or tail paint being removed on days 22-24. All inseminations over the 4 year period were carried out by the same experienced technician. Pregnancy diagnosis was carried out by ultrasound around day 35 after AI.

Results: Breeding results from the double synchronisation programme over a 4 year period are summarised below.

First service conception rate (CR) to TAI : 65% (63/97); 67% (102/152); 66% (103/156); 68% (106/156). Mean CR over 4 years 67% (374/561).

Second service CR to observed heats/AI : 59% (13/22); 41% (12/29); 36% (13/36); 58% (18/31). Mean second service CR 47% (56/118).

Cow pregnancy rate after 24 days breeding : 78% (76/97); 75% (114/152); 74% (116/156); 79% (124/156). Mean pregnancy rate after 24 days 77% (430/561).

The P implants appeared to have enough residual P to prevent early returns of non-pregnant cows prior to day 21. The re-use of CIDR implants after washing/disinfection or autoclaving has been described by various authors however is not a licensed use of the product. In the herd described above, used CIDR implants were only used for the 5 day re-synchrony period and were not considered suitable for primary synchronisation.

Conclusions: The synchronisation protocol delivered consistent results (mean 67% CR) over 4 years showing that, under good management, beef cows can be highly fertile at first service after calving when bred to TAI. Resynchronising cows after TAI using P implants can allow a targeted period of heat detection to allow a second round of AI. In this herd the CR to the second round of AI was acceptable at 47% which allowed 77% of cows to be successfully impregnated by AI in a 24 day breeding period. Double synchronisation may be a practical method to breed beef cows by AI with minimal heat detection and could be followed by sweeper bull mating.

Reproduction: (Dairy)

P03-003-181

Effect of Calving Difficulty on Reproductive Performance and Functional Longevity in Holstein Cows

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Objectives: The objective of this study was to evaluate the effect of dystocia on subsequent reproductive performance and functional longevity in Iranian Holsteins.

Materials and Methods: Data consisted of 1,467,064 lactation records of 581,421 Holstein cows from 3083 herds which were collected by the Animal Breeding Center of Iran from April 1987 to February 2014. Reproduction traits in this study included interval from first to second calving, days open and days from first calving to first service. Calvings were scored on a 5-point system of difficulty with increments of 1, where score 1 = unassisted, score 2 = slight assistance, score 3 = considerable assistance, score 4 = considerable force needed, and score 5 = caesarian. The generalized linear model was used for the statistical analysis of reproductive traits. Survival analysis was performed using the Weibull proportional hazards models to analyze the impact of dystocia on functional longevity.

Results: The incidence of dystocia had an adverse effect on the reproductive performance of dairy cows. Therefore, reproductive traits deteriorated along with increase in dystocia score (P<0.05). The culling risk was increased along with increase in the score of dystocia (P<0.0001). The greatest culling risk was observed in primiparous cows, small herds and low- yielding cows (P<0.0001). Also, the lowest culling risk was found for cows calving at the youngest age (<27 months) and cows with age at first calving >33 months had the greatest risk (P<0.0001).

Conclusions: The results of current study indicated that dystocia had important negative effects on the reproductive performance and functional longevity in dairy cows and it should be avoided as much as possible to provide a good perspective in the scope of economic and animal welfare issues in dairy herds.

Reproduction: (Dairy)

P03-003-182

Fertility after different resynchronization protocols with timed AI in 32 commercial dairy farms

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Objectives: After first inseminations an average of at least 60% of the cows are usually not pregnant, such that strategies to achieve pregnancy of these cows as soon as possible are very important for profitability of the farms. Different resynchronization protocols using prostaglandin (PG) and GnRH with timed artificial insemination (TAI)

have been designed. The mean objective is to maximize the pregnancy rate, sharply influenced by the interval AI-pregnancy diagnosis.

The aim of this study was to evaluate fertility and pregnancy rate of resynchronization protocol.

Materials and Methods: Data of 4279 inseminations of cows coming from 32 different commercial farms distributed in the East region of Spain were included. The average farm size was 400 lactating cows (range between 90 and 1400 cows/farm) and 10.507L/cow/lactation. The resynchronization protocols compared were G6G (n=3574 AI; PG at day 0, GnRH at day 2, day 8 GnRH, day 15 PG, day 15 PG; day 16 p.m. GnRH and TAI at day 17) versus 5days-Ovsynch (5DO; n=705 AI; GnRH at day 0; PG at days 5 and 6; day 7 p.m. GnRH and TAI at day 8). Risk factors included were parity (primiparous vs. multiparous) and season at TAI (hot vs. cool season).

Results: Farm significantly affected fertility (P<0,001) ranging between 17-59% with an averaged fertility of 36.4±12.9% and included as factor in the regression model. Parity did not show a significant influence on the fertility (36.42±12.39 vs. 36.4±13.8% for multiparous and primiparous respectively; P>0.05). On the other hand, season and synchronization protocol significantly affected fertility. During the hot season the fertility was 23.5±8.7% and during the cool season it was 40.8±11.0% (OR=2.241; P<0.001). Finally, the fertility of inseminated cows after G6G was 38.1±12.3 and after 5d-Ovsynch 26.5±12.2% (OR=1.477; P<0.001). When parity was conserved in the model, the difference in fertility in multiparous showed the same effect (G6G was significantly better than 5d-Ovsynch), however, in primiparous, this difference showed only a statistical tendency to be better with G6G versus 5d-Ovsynch (38.0±12.8 vs. 31.1±15.4%; P=0.06).

The difference between TAI-TAI in the protocols is essential to decide the best strategy to obtain the best pregnancy rate. Given an interval for pregnancy diagnosis of 31d, the insemination rate with G6G-protocol (interval TAI-TAI of 18d and subsequent insemination rate of 43%) and the fertility of 38% gave a pregnancy rate of 16%. With the 5d-Ovsynch protocol, the TAI-TAI interval of 9d gives an insemination rate of 50%. This would allow a fertility as low as 32% to get the same pregnancy rate than with the G6G. With larger interval AI-Pregnancy diagnosis, the fertility allowed for 5d-Ovsynch can be reduced to get the same pregnancy rate than with G6G.

Conclusions: G6G fertility was higher than that after 5d-Ovsynch protocol, but the interval TAI-TAI reduction of the shorter protocol allows a lower fertility to get the same pregnancy rate at a fixed interval AI-pregnancy diagnoses. These factors should be considered, when a decision has to be taken.

Reproduction: (Dairy)

P03-003-183

Associations between the intrauterine cytobrush procedure in dairy cows and purulent vaginal discharge, reproductive performance and milk production

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Objectives: Reproductive tract inflammation and infection have a negative impact on reproduction performance and there are a number of diagnostic tools available to assess those diseases. Cytology using cells recovered by transcervical placement of a human pap-smear brush

has been a frequently used method in dairy reproductive research in the last 10 years. The objective of this study was to assess the associations between the use of the cytobrush and subsequent diagnosis of purulent vaginal discharge (PVD), reproductive performance, and milk production.

Materials and Methods: Dairy cows (n=272) from 6 spring-calving dairy herds in the Waikato, New Zealand were assessed at 3 time points postpartum; D0 (median: 2; range: 1-7 days in milk), D21 (±0), and D42 (±2). On D0 and D21 two intrauterine samples were collected via triple guarded cytobrushes. The first cytobrush was used for bacteriological assessment of *E. coli* and *T. pyogenes*. The second cytobrush was used to prepare a cytology slide. On D42, PVD was evaluated on a 0 to 5 scale (>1 defined as PVD positive) using a Metrichheck device™, (Simcrotech, Hamilton, New Zealand). Milk production at one time point was assessed (median interval between seasonal planned start of calving to herd milk production test=120 days; range=91-141 days). Milk production was categorized in low milk solids (MS; ≤mean MS for each herd - SD), medium MS (>mean MS for each herd - SD to ≥mean MS for each herd + SD) and high (>mean MS for each herd + SD). Mean MS minus & plus SD for herd 1 were 1.41 & 2.19 kg MS, respectively, and 1.43 & 2.21, 1.55 & 2.44, 1.43 & 1.87, 1.16 & 1.53 and 1.62 & 2.18 kg MS for herds 2, 3, 4, 5, and 6, respectively. Cows assessed with the cytobrush procedure were matched 1:2 by herd, age (categorized in 3 and 4, 5 and 6, and >6 years old) and breed (Friesian, Jersey, and Cross breed) with cows that were not sampled. Associations were examined using multivariable conditional logistic regression for matched studies.

Results: The cytobrush procedure increased the odds of PVD at a median of 44 days post-partum, with 20/265 (8%) cases and 17/530 (3%) controls having PVD, respectively (OR=4.05 (95%CI= 1.81-9.05); P=0.001). Interestingly, the procedure was associated with increased odds of pregnancy by 42 days after the start of the seasonal breeding programme (planned start of mating; PSM), with 155/265 (58%) cases and 230/530 (43%) controls pregnant at 42 days after PSM, respectively (OR=1.55 (95%CI= 1.15-2.33); P=0.01).

Additionally, with low milk production as the reference, cases were more likely to have a medium milk production compared to controls, with 31/263 (12%) cases and 81/528 (15%) controls having a low production, 206/263 (78%) cases and 360/528 (68%) controls having a medium production, and 26/263 (10%) cases and 87/528 (16%) controls having a high production, respectively (OR=1.83; P=0.02 and OR=0.95; P=0.90 for medium and high milk production, respectively).

Conclusions: The cytobrush procedure to detect uterine inflammation and intra-uterine presence of bacteria increased the probability of having and being treated for PVD 44 days post-partum. Interestingly, the cytobrush procedure was also associated with increased probability of conceiving in the first six weeks after PSM and with increased probability of having a medium milk production compared to control cows.

Reproduction: (Dairy)

P03-003-184

Treatment Of Puerperal Metritis In Dairy Cows Using Two Antibiotics Combined Or Not With A Prostaglandin F2alpha Analogue

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Objectives: Uterine infections are one of the main causes of reduced fertility in dairy cows. Puerperal infections are frequent after dystocia and retained placenta. Besides the negative impact in fertility, uterine infections can eventually cause cattle death; thus, effective treatments are important to avoid economical losses. The objective of this study was to evaluate the efficacy of two antibiotics, associated or not with sodium cloprostenol (SC), in the treatment of puerperal infections in dairy cows.

Materials and Methods: Holstein cows (N=100) showing retained placenta 18 to 36 hours after birth were selected for this study. These cows were randomly allocated in 4 treatment groups, balanced according to parity and body condition score, in a factorial 2x2 design (2 antibiotics, combined or not with SC). The treatments were given im at the first day after calving as follows: T1 (n = 25), cefquinome (Cobactan, MSD Saude Animal, Sao Paulo, Brazil), 1mL / 25kg body weight (BW) once a day for 3 days; T2 (n = 25), cefquinome in the same protocol of T1 plus 0.5 mg of SC (Ciosin, MSD) at the first and third days of treatment; T3 (n = 25), oxytetracycline hydrochloride (Terramycina LA, Zoetis, Sao Paulo, Brazil) for two days at 48-h interval; and T4 (n = 25), oxytetracycline in the same protocol of T3 plus 0.5mg SC on the first and third days of treatment. Rectal temperature was measured daily for five days postpartum. The cows were evaluated between the third and fifth weeks postpartum by rectal palpation, ultrasonography, and vaginoscopy, to assess the presence of uterine infection (endometritis). We evaluated the interval from calving to first service, the conception rate at the first insemination, the number of pregnant cows up to 250 days after parturition, the average number of services per conception, and the number of days open (Tukey test). A p-value of 0.05 indicated statistical significance.

Results: The percentage of cows showing hyperthermia (temperature >39.0°C) between the second and the fifth day postpartum was 12.0%^a; 8.0%^a; 40.0%^b and 24.0%^{ab} for T1, T2, T3, and T4, respectively (P<0.05). The percentage of cows diagnosed with endometritis between the third and fifth weeks postpartum was better in cefquinome groups (36.0%^a; 24.0%^a; 64.0%^b and 48.0%^{ab} for T1, T2, T3, and T4, respectively - P<0.05). The interval from calving to first service (67.7±18.6^b, 53.2±13.8^a, 74.0±22.7^c, and 64.7±15.1^b days), the percentage of pregnant dairy cows up to 250 days postpartum (76.0%^{bc}; 88.0%^c; 56.0%^a, and 68.0%^{ab}), and the number of days open (158.5±86.4^b, 144.6±72.0^a, 179.8±96.2^c, and 161.3±81.2^b days) differed between treatments T1, T2, T3, and T4, respectively (P<0.05). However, there was no difference in the conception rate at the first AI nor in the number of services per conception among treatments (24.0%; 32.0%; 16.0%, and 20.0% and 3.2±1.8, 2.9±1.5, 3.5±2.0, and 3.3±1.9 for T1, T2, T3, and T4, respectively; P>0.05).

Conclusions: Cefquinome is more efficient than oxytetracycline hydrochloride in the treatment of puerperal metritis in dairy cows with retained placenta. Prostaglandin treatment of cows with retained placenta and subsequent uterine infections, improved fertility by reducing the number of days open, independent of the antibiotic used.

Comments: Thanks: Fapemig, CNPq and CAPES

Reproduction: (Dairy)

P03-003-186

Fixed-time artificial insemination following progesterone-based synchronization protocols associated with GnRH or estradiol in Holstein heifers

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Objectives: Due to the low cost and good results in pregnancy rates, administration of estrogen, concurrently with a progesterone device, is the most widespread hormonal protocol used in Brazil for fixed-time artificial insemination (FTAI) in beef and dairy cattle. However, legal restrictions of the use of estrogens in animal products should limit the use of these substances in the near future. The aim of this study was to evaluate the conception rates of Holstein heifers treated with an alternative protocol (5-day CO-Synch + CIDR) to the conventional 8-day progesterone-based synchronization protocol associated to estradiol.

Materials and Methods: Pubertal Holstein heifers (n=143) were treated (Day 0) with a progesterone intravaginal device (Primer®, Tecnopec, São Paulo, Brazil) containing 1 g of progesterone and assigned randomly in two groups. Group 1 (n=75) was injected with GnRH (100µg i.m. Cystorelin, Merial, Duluth, GA, USA) and group 2 (n=68) with estradiol benzoate (2mg EB, Estrogin, Farmavet, São Paulo, Brazil). Primer was removed on Day 5 (group 1) or Day 8 (group 2) and administered one injection of cloprostenol (125 mcg, Prolise®, Tecnopec, São Paulo, Brazil). Twenty-four hours later, Group 2 received 1 mg EB. Estrus was monitored (Estrotec®, Rockway, Inc. USA) after cloprostenol injection and FTAI (semen from one sire) 72 (Group 1) or 54 (group 2) hours after Primer removal, and GnRH was administered concurrent with FTAI in Group 1. Heifers from group 1 showing estrus until 54 hours after cloprostenol injection were inseminated at the end of estrus. Pregnancy status was evaluated by transrectal ultrasonography on day 40 after FTAI. The effect of treatments in pregnancy rates after FTAI was analyzed by the GLIMMIX procedure of SAS. The variables included in the model were: age, weight, estrus manifestation, follicle size at time of insemination and interaction of the main effects.

Results: The pregnancy rate was not affected by age, weight, follicle size or estrus manifestation (P>0.05). Most heifers of group 2 (88.2%) than group 1 (33.3%) were in estrus at FTAI (P<0.05). On the other hand, 40% of heifers of group 1 showed estrus in the 24 hours to 54 hours period after cloprostenol injection. The pregnancy rate of heifers inseminated at standing estrus in this period was 50.0% vs 48.9% at FTAI (P>0.05). At all, pregnancy rate of groups 1 (IA + FTAI) and 2 (only FTAI) were 49.3 and 51.5%, respectively (P>0.05).

Conclusions: Similar pregnancy rates can be achieved with the 5-day Co-synch and BE protocols. However, considering the high proportion of heifers of group 1 that were inseminated on standing estrus in the period < 54 h after injection of cloprostenol, further studies are necessary to elucidate if co-synch protocol can be considered adequate to FTAI.

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Objectives: Identification of open cows and their enrollment in a suitable synchronization protocol is an essential part of improved reproductive management in any dairy enterprise. The objectives of the study were to appraise the controlled internal drug release (CIDR) based protocols to synchronize estrus followed by resynch and to compare their fertilizing ability through fixed time artificial insemination (FTAI) in postpartum dairy cows at two different geographical locations

Materials and Methods: In Experiment 1 (location A), postpartum cows (n=160) were assigned to receive standard CIDR-EB (estradiol benzoate) synchronization protocol. Cows were inseminated artificially, using frozen thawed semen, 48-60 hours after CIDR removal and randomly assigned into two groups: 1) RESYNCH group (n =90) received CIDR for d14-d21 post TAI. On day 23rd, all the cows received 100 µgGnRH (Dalmareline, Fatro Italy; i.m.) while their pregnancy was diagnosed on d30 through ultrasound (US). Open cows in RESYNCH group received PGF2α (Dalmazin, Fatro Italy; i.m) and GnRH following standard ovsynch protocol. Pregnancy was confirmed and reconfirmed on d60 and d90, respectively. Blood sampling was done on day 14, 16 and 30 for P4 analysis. 2). CONTROL group (n =70) subjected to AI after detected estrus from 18-30 days post TAI. PD timeline was same as in RESYNCH group. Corpus luteum (CL) cross-sectional area was measured on d30 in pregnant animals in both RESYNCH (n=30) and CONTROL (n=27) group. In experiment 2, both RESYNCH (n=54) and CONTROL (n=64) groups were similarly treated but without blood sampling and CL area measurement

Results: Effect of CIDR based synchronization and RESYNCH treatment on first-service conception rates and overall pregnancy rates were determined by chi square analysis in PROC FREQ of SAS. Effect of treatment on circulatory P4 profile and luteal tissue cross sectional area were analyzed by using GLM procedures of SAS. Differences were declared significant if p< 0.05. In experiment 1, pregnancy rate on d30 between RESYNCH (48%) and CONTROL (43%) groups did not differ statistically (p=.50). On d60 and d90 overall pregnancy rate was 73 % and 44% (P = 0.002) and 72 % and 43% (P = 0.002) in RESYNCH and CONTROL groups, respectively. Overall pregnancy loss in CONTROL (11%) group was higher in comparison with RESYNCH (4%) group. In experiment2, pregnancy rate on d30 in RESYNCH (54%) and control (44%) groups did not differ statistically (p=0.28). Overall pregnancy rates on d60 and d90 were 72 % vs. 70% and 53% vs. 50% in RESYNCH and CONTROL (P = 0.03; P = 0.025) groups respectively with overall pregnancy loss in RESYNCH (3%) and control groups (14%) group. Mean plasma P4 profile (ng/mL) was measured in control versus RESYNCH group on d14 (5.50± 1.12 vs.5.38± 0.96; p=0.53), d16 (6.47± 0.99 vs. 5.60 ± 1.15; p=0.0036) and d30 (7.57± 1.14 vs. 6.34± 1.32; p=0.0002) post TAI. Mean luteal cross-sectional area on d30 post TAI was measured in both RESYNCH and CONTROL groups was 422±98 and 490± 127mm²; p= 0.03). Other parameters including BCS, cyclicity and parity did show any impact on pregnancy neither independently nor in interaction with treatment

Conclusions: In conclusion, we observed a significant increase in overall pregnancy rate in RESYNCH group in comparison with CONTROL. CIDR based RESYNCH protocol has resulted in significantly higher conc. of P4 despite of small luteal tissue area as compared to control group on d30 post TAI.

Reproduction: (Dairy)

P03-003-187

Effect of resynchronization on already synchronized postpartum dairy cows

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Reproduction: (Dairy)

P03-003-188

Administration of oral calcium in cows diagnosed with toxic puerperal metritis: Cure and subsequent incidence of periparturient health disorders

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Objectives: The objective was to evaluate the effect of oral calcium administration on the cure, survival, reproductive performance, and subsequent presentation of periparturient health disorders (clinical and subclinical mastitis, pneumonia, and lameness) of Holstein cows diagnosed with toxic puerperal metritis (TPM) in an organic dairy farm in Texas, USA. A secondary objective was to analyze the effect of metabolic status at calving (calcium and nonesterified fatty acids [NEFA] serum concentrations) on the incidence of TPM and other diseases during early lactation (clinical and subclinical mastitis, pneumonia, and lameness).

Materials and Methods: Cows with TPM were defined as having an enlarged uterus and a fetid watery red-brown vaginal discharge, associated with systemic illness and fever, within 12 d postpartum. Cows diagnosed with TPM (n=200) were blocked by parity and randomly assigned into 1 of 2 intrauterine treatments applied every other day for a total of 3 treatments: i) Control (CON)=Optimum UterFlush® (Van Beek Natural Science, USA) =3.75 mL diluted in 117 mL of distilled water (n=100); ii) Calcium supplemented (CA)=Same intrauterine treatment plus 6 calcium oral capsules ('O' Cal-D Cap™, Bio-Vet Inc., USA; Ca 7,500-9,000 mg/capsule) once per day, for 3 days. All enrolled cows received hypertonic saline solution (500 ml 7.2% i.v.), dextrose (500 ml 50% i.v.), and oral aspirin (5 boluses/d). Outcome variables included fever, presence of fetid vaginal discharge, and cervix diameter at study d 6 and d 14, clinical endometritis at 32 DIM, survival at 30 and 100 DIM, reproductive performance, and incidence of health disorders after TPM recovery. A group of 200 healthy cows was matched to the enrolled cows (by parity, DIM, BCS) and calcium and NEFA serum levels were determined at calving and at the time of enrolment. Calcium was also measured in the TPM cows at d 1, 2, 3, and 6 post diagnosis. Control variables were parity, BCS at enrolment, and d at diagnosis. Differences between treatment groups were tested by logistic regression, repeated measures analysis, and ANOVA. The significance level for inclusion in the final model was set at P ≤ 0.10. Significance was set at P ≤ 0.05.

Results: No difference was found for Calcium treatment effect on fever, presence of fetid vaginal discharge, and cervix diameter at study d 6 and d 14. Similarly, clinical endometritis at 32 DIM and survival (30 and 100d) were not affected by treatment. However, the proportion of cows bred by 200 DIM was affected by Calcium treatment (66% vs. 55% for CA and CON groups, respectively [P < 0.05]). Serum concentrations for calcium at d 1, 2, 3, and 6 post diagnosis was significantly higher in the CA treatment. The odds of TPM were significantly higher for cows with low calcium levels at calving (Ca < 8 mg/dL; odds ratio [95%CI] = 2.07 [1.27-3.36]). Similarly, the odds of TPM for cows in severe negative energy balance (NEFA >0.5 mmol/L) were 3.3 (2.03 – 5.35) times the odds of TPM for healthy cows. At the day of TPM diagnosis serum calcium was lower in TPM cows compared with healthy cows (6.29 vs. 8.39 mg/dL; P < 0.001). Similarly, NEFA serum concentrations were

higher in TPM cows compared with healthy cows (0.49 vs. 0.35 mmol/L; P < 0.001). Finally, the odds of pneumonia for cows in severe negative energy balance were 3.86 (1.1 – 14.7) times the odds of pneumonia for healthy cows.

Conclusions: Supplementing oral calcium to the treatment of toxic puerperal metritis had no effect on clinical cure or survival, but resulted in a higher proportion of cows bred at 200 DIM. Hypocalcemia and high NEFA levels at calving were significant risk factors for occurrence of TPM and cows affected with TPM had lower calcium and higher NEFA concentrations at the time of diagnosis.

Reproduction: (Dairy)

P03-003-189

Addition of eCG to a G7G protocol: effects on ovarian dynamics in lactating dairy cows in New Zealand pastoral system

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Objectives: 1) To observe and record the ovarian response of anoestrus and cycling lactating dairy cows to a G7G synchrony protocol, and 2) to evaluate the effect of the addition of eCG to the final prostaglandin injection of a G7G synchronisation program on follicle dynamics and luteolysis in lactating dairy cows.

Materials and Methods: After balancing for cyclicity status (anoestrus vs cycling) at initiation of treatment based on ultrasound examination, 64 lactating cows were treated intramuscularly as follows: on Day -17 (Day 0 = timed-AI) cows were treated with 100 µg of GnRH (pre-G, Ovurelin) and 500 µg of sodium cloprostenol (pre-P; PG, Ovuprost). Seven days later (Day -10), cows were given GnRH (1G; first treatment of Ovsynch) followed on Day -3 by a dose of PG plus or minus 400 i.u. of eCG (Pregnecol reconstituted in Ovuprost, n=32/group). A final dose of GnRH was administered 48 hr later (2G, Day-1) and timed-AI was scheduled 16-20 hours after 2G (Day 0). Animals detected in heat between Days -3 and 0 were inseminated 12 h later (am/pm rule) and were removed for further analysis. Twice daily ultrasound examinations were conducted from Day -3 until timed-AI and repeated 24 h later for detection of ovulation. Progesterone (P4) concentration was assessed on Days -3 and 0 (at timed-AI) in blood samples collected via coccygeal venepuncture.

Results: On Day -3, 95.3% of cows had luteal tissue at ultrasonography (14/17 and 47/47 of anoestrus and cycling cows, respectively) and mean P4 concentration was 5.0±0.53 ng/mL (2.6±0.56 and 5.4±0.52 ng/mL in anoestrus and cycling cows, respectively; P<0.01). Fifty-five percent of cows (35/64) had at least two CLs on Day-3, which was considered indicative of ovulatory response to the pre-G/pre-P treatment (41% and 60% in anoestrus and cycling cows, respectively; P=0.26). No effect of treatment, time and their interaction was detected on day-to-day dominant follicle and CL diameter profiles and 59 of 61 cows underwent morphologic luteolysis, defined as progressive and sustained decrease on CL diameter over 4 consecutive examinations. Six cows (two in G7G and four in G7G+eCG) showed signs of oestrus prior to timed-AI and were inseminated earlier. On Day 0, blood samples were obtained from 48 of 53 cows presented for timed-AI. Mean P4 concentration at AI was 0.3±0.03 ng/mL (0.3±0.03 and 0.4±0.05 ng/mL in G7G and G7G+eCG, respectively). Two out of 25 and two out of 23 cows in the G7G and G7G + eCG groups had P4 concentrations ≥0.6 ng/mL at

timed-AI. Percentage of cows that ovulated to the 2G administration was 96.2% (51/53). Pre-ovulatory dominant follicle diameter was 19.8 ± 0.41 and 19.5 ± 0.63 mm in G7G and G7G+eCG, respectively. Overall, the percentage of synchronised animals (with CL at ultrasonography on Day-3, undergoing morphologic luteolysis and ovulating synchronously after 2G treatment) were 84.4% (27/32) and 75% (24/32) in the G7G and G7G+eCG groups, respectively ($P=0.54$).

Conclusions: Acceptable ovulation rates and progesterone concentrations in cycling and anoestrus cows synchronized with a G7G program could eliminate the need for detection of cyclicity prior to the planned start of mating. Addition of eCG reconstituted in the PG solution did not affect the ovarian dynamics measured in this trial. Although pending at submission of this abstract, the pregnancy rate results from this study and from a larger study run in parallel, will be pivotal to validate whether addition of eCG to the G7G program has positive effects on fertility in lactating dairy cows, overcoming the effect of ovarian status at initiation of treatment.

Reproduction: (Dairy)

P03-003-190

Effects of prolonged consumption of water with elevated nitrate levels on body condition score and certain reproductive parameters of dairy cattle

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Objectives: Water is the most common source of nitrates in non-grazing animals. Ingested nitrates are converted to nitrites and then to ammonia by rumen flora. As this mechanism overloads, nitrite builds up and enters blood stream. Alterations on certain metabolic pathways, due to increased ammonia production and/or nitrite absorption could impair dairy cattle productivity in chronic subclinical nitrate toxicity. The objective was to evaluate the effects of prolonged consumption of water naturally contaminated with nitrates on body condition score and certain reproductive parameters and whether dietary inclusion of clinoptilolite could ameliorate these.

Materials and Methods: Two experiments were run simultaneously for the purpose of the study. In the first experiment (Exp.1) farms 1 and 2 were assigned into two groups according to the nitrate levels in the borehole water of the previous year. The first group (NG) consisted of the farm 1 (milk yield; MY 35 kg/cow/day) that had nitrate levels >40 ppm and the second one (CG) of the farm 2 (MY 36 kg/cow/day) that had water nitrate levels <40 ppm. In the second experiment (Exp.2) farms 3 and 4 were assigned into two groups according to the nitrate levels in the borehole water and the use of clinoptilolite as follows: the first group (NC) consisted of the farm 3 (MY 31 kg/cow/day) that had nitrate levels >40 ppm and used clinoptilolite as feed additive at the rate of 2.5% of concentrates. The second one (CG) consisted of the farm 4 (MY 29 kg/cow/day) that had water nitrate levels <40 ppm without adding clinoptilolite in the ration. Both experiments lasted 6 months. Each farm was visited 3 times, at the onset of the experiment and then at 3-month intervals. At each visit, 15 clinically healthy dairy cows, 5 in early lactation (<150 days in milk; DIM), 5 in late lactation (≥ 150 DIM) and 5 dry cows were selected randomly for body condition scoring (BCS). Certain reproductive parameters (calving to first service interval and number of services per conception) from all cows that delivered and

received at least one insemination during the study period, as well as the data concerning the nitrate levels at the borehole water were obtained from the records of the farms. The data was analyzed with SPSS® v. 21.

Results: The average water nitrate levels during the experimental period were 87 ± 12.5 ppm for farm 1, 4 ± 1.5 ppm for farm 2, 13 ± 2.4 ppm for farm 3 and 76 ± 14.2 ppm for farm 4. The reproductive data of 69 cows (36 in NG and 33 in CG) in Exp. 1 and 50 (23 in NC and 27 in CG) in Exp. 2 were used in the study. BCS was unaffected by prolonged nitrate consumption in both experiments ($P>0.05$). Calving to 1st service interval tended to be significantly higher in nitrate groups in both experiments (Exp. 1: 121.5 ± 7.68 and 102.5 ± 7.25 for groups NG and CG, $P=0.078$; Exp. 2: 114.3 ± 6.45 and 98.5 ± 5.33 for groups NC and CG, $P=0.063$). The number of services per conception was significantly higher in NG compared to the CG (2.21 ± 0.15 and 1.77 ± 0.14 for groups NG and CG, $P<0.05$) in Exp. 1, but no significant difference was detected between groups NC and CG in Exp. 2 (1.71 ± 0.14 and 1.63 ± 0.13 for groups NC and CG, $P>0.05$).

Conclusions: The results obtained provide evidence for possible impairment of the reproductive efficiency of dairy cattle that consume water with high nitrate levels for a prolonged period of time. These effects can be partially controlled by the dietary administration of clinoptilolite.

Reproduction: (Dairy)

P03-003-191

Influence of Intravenously Glucose Infusions in Pre-estrus on Cycle Length and Further Parameters of the Estrus Cycle in Lactating Dairy Cows

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Objectives: Delayed ovulation is one of the reproduction disorders in lactating dairy cows which subsequently influences conception rate. Among others a significant positive correlation between daily milk yield and the incidence of delayed ovulations has been demonstrated. The objective of this study was to test whether an energy supply by intravenously administered glucose infusions at different days in pre-estrus affects cycle length, size of the preovulatory follicle, plasma concentrations of progesterone and estradiol-17 β and expression of estrus behavior in dairy cows beyond negative energy balance after parturition.

Materials and Methods: Follicular development and time of ovulation were documented in ten multiparous lactating Holstein Friesian dairy cows by means of daily transrectal sonography (8 MHz transrectal probe, LCS Scanner 100®, Pie Medical, Maastricht, NL). Blood samples were taken once daily starting day 17 of the estrus cycle to determine concentrations of glucose, progesterone and estradiol-17 β until day 5 after ovulation. In every cow three unmanipulated cycles (group 1; control; n = 30) and three cycles after infusion of 1000 mL 5% glucose solution on day 18 (group 2; n = 10), 19 (group 3; n = 10) and 20 (group 4; n = 10) were investigated.

Results: Glucose infusion on day 19 (group 3) resulted in a significant shortening of the cycle length compared to controls (21.3 ± 1.3 vs. 23.3

± 1.9 days; $p < 0.01$). Treatments on the 18th (23.0 ± 1.8) and the 20th day of the estrus cycle (22.6 ± 1.6) had no significant effect, respectively. Treatment did not influence maximum diameter of the preovulatory follicle ($p > 0.05$), but it varied significantly in between the ten individual cows ($p = 0.018$). There was a trend to detect more cows in heat after intravenously glucose administration on day 19 (56.7 % of cycles in group 1 vs. 90 % in group 3) but this difference could not be proven significantly ($p = 0.12$).

Conclusions: Even though the negative correlation between energy status and fertility in high-yielding dairy cows is well established nearly all studies focus on the period of negative energy balance after parturition. These results show that also beyond this period a short-term supply of energy at the beginning of pre-estrus has a significant effect on time of ovulation.

Reproduction: (Dairy)

P03-003-192

Influence of Preovulatory Glucose Infusions on the Secretion of Luteinizing Hormone and the Time of Ovulation in Lactating Dairy Cows

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Objectives: One of the reasons for the decline in reproductive efficiency of dairy cows is disorders in the ovulatory process like e.g. delayed ovulation. Reports about its incidence vary between 17 – 46 % of the examined cows. An insufficient energy supply at the time of ovulation may contribute to this effect. In a preceding study we demonstrated a shortening of the estrus cycle length by intravenous infusion of glucose at day 19. The aim of the present study was therefore to test the effect of this treatment on the preovulatory secretion of Luteinizing Hormone (LH), estradiol 17β , progesterone and glucose.

Materials and Methods: The ovaries of ten multiparous lactating Holstein Friesian cows, which were kept in tie-stalls, were examined every two days and from a diameter of the dominant follicle over 10 mm on daily via transrectal sonography (8 Mhz transrectal probe, LCS scanner 100, Pie Medical®, Maastricht, NL) for patterns of follicular growth and time of ovulation. From day 17 of the estrus cycle until day 5 after ovulation blood samples were collected to determine serum concentrations of progesterone, estradiol- 17β and glucose. Cows were randomly allocated into two groups: In group 1 ($n = 5$) cows received an intravenously infusion of 1000 mL of a 5% glucose-solution and in group 2 ($n = 5$) cows received an infusion of 1000 mL 0.9 % saline-solution on day 19 of the estrus cycle. Blood samples for the determination of the preovulatory LH-concentrations were taken every three hours from the end of the treatment until ovulation.

Results: Mean length of estrus cycles was shorter in group 1 compared to group 2 (20.2 ± 1.1 days vs. 21.8 ± 0.8 days; $p = 0.18$). The LH-peak-concentrations differed significantly between the two groups ($p = 0.043$), being much lower in group 1 after glucose infusion (geometric mean 12.27 ng/ml) than in group 2 (control; geometric mean 34.36 ng/ml). The interval between infusion and LH-peak was distinctly shorter in group 1 (25.8 ± 9.6 hours) than in group 2 (52.8 ± 35.7 hours; $p = 0.21$), whereas the intervals between LH-peak and ovulation did not differ between

groups. No significant differences in the concentrations of glucose could be detected.

Conclusions: A short-term supply of glucose on day 19 of the estrus cycle shortens the cycle length by an earlier induction of the preovulatory LH-peak. It is well known that the application of glucoprivic drugs (like 2-deoxy-D-glucose) rapidly suppresses LH-pulses in rodents and in sheep. Thus LH-secretion responds directly to changes in metabolic fuel oxidation. Also in dairy cows the preovulatory LH-secretion seems to be influenced directly by the availability of glucose.

Reproduction: (Dairy)

P03-003-193

Relationship between scrotal circumference, weight, growth, age, attainment of puberty and Australian Breeding Values in pasture based dairy bulls.

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Objectives: Natural service sire use post artificial insemination in dairy herds in South Eastern Australia is common. The bull breeding soundness exam (BBSE) provides a tool to assess the risk of subfertility in bulls and whilst it has been extensively used in the beef industry, its use in dairy is increasing.

In order to inform the use of the BBSE in dairy bulls this project aims to collect data on scrotal circumference and weight in growing dairy breeds. Furthermore, semen collections will monitor attainment of puberty. Possible links between scrotal circumference, weight and Australian Breeding Values will also be investigated.

Materials and Methods: The scrotal circumference and live weight of two groups of growing pasture based bulls were monitored at 6 weekly intervals. The groups comprise the two major dairy breeds used in the area, Jersey ($n=85$) and Holstein-Friesian ($n=145$).

Semen samples collected via electroejaculation were taken around the time of puberty as predicted by scrotal circumference. Holstein-Friesian bulls have been determined to reach puberty at a scrotal circumference of 28cm. In order to capture data before and after this stage semen sampling was instituted when bulls achieved a scrotal circumference of 25 cm. Semen samples will be assessed for morphological evidence of maturity (percentage live sperm and presence of proximal droplets).

Pedigree and sire information will be collected and hair samples taken from each bull for genomic testing (Clarifide, Zoetis) to determine the Australian Breeding Values for each bull. The resulting values will then be analysed for potential relationships between scrotal circumference and daughter fertility.

Results: Preliminary results show positive correlations between growth and scrotal circumference for both groups of bulls. There may be some differences between breeds, however, data collection continues and full analysis is yet to be obtained.

Genomics information is yet to be assessed; however areas of interest include the potential relationship between Australian Breeding Values, particularly scrotal circumference and daughter fertility. There may be potential value in adding genomics evaluations to the standard BBSE for natural service sires.

Conclusions: The BBSE is an increasingly important tool for veterinarians and producers in assessing the fertility risk of natural service sires in the dairy industry. In collating more information on dairy breed sires we hope to enable this decision making tool, resulting in better fertility and improved stock selection both for producers and the industry at large.

Comments: My submission could be considered 'young stock' or 'dairy herd health management' as well as 'reproduction- dairy'.

Reproduction: (Dairy)

P03-003-194

Comparison Of A Novel Ovsynch Program Utilising Early Pregnancy Diagnosis As A Measure Of Performance In Australian Pasture Based Dairy Herds

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Objectives: This study aims to compare a new ovsynch (ovulation synchronisation) program that incorporates a second prostaglandin injection (PGF_{2α}) with an existing ovsynch program to determine whether or not an improvement in conception rate can be achieved in pasture based, seasonal herds in Australia. This study will also examine the progesterone levels of a subset of cattle as an indication of whether ovulation is occurring at the time of insemination in both groups. After pregnancy diagnosis has occurred, any animal that was not pregnant and was not detected in oestrus post insemination will be examined retrospectively and available data analysed.

Materials and Methods: Two experiments were performed using two groups of predominantly Holstein-Friesian cows which were chosen using the odd and even numbers on their eartags to randomly assign them to the two groups. The cows used were both primiparous and multiparous lactating animals. These animals were sourced from several farms in South West Victoria, Australia. Group 1 was given the original OVSYNCH program which incorporated a GnRH injection at day 0, one PGF_{2α} injection at day 7 and a GnRH injection 56 hours later followed by fixed time artificial insemination 16-20hours after the final GnRH. Group 2 was given the modified ovsynch program which incorporated a second PGF_{2α} injection on day 8. A subset of animals, randomly chosen based on their eartags from both groups, were subject to two blood tests on day 7 and 10, to measure progesterone levels. This will be an indication of whether the animals ovulated at the time of AI and determine if there was a significant difference between the two groups of animals. All animals were submitted for early pregnancy diagnosis between day 35 and 40 after AI, using linear ultrasound, and the results recorded. The Data will be analysed to determine whether there was a statistically significant difference in pregnancy rate between the two ovsynch programs and if there was an improvement in the conception rate using the modified approach.

Results: This experiment is not yet complete and will involve about 3000 cows (n = 1500 in each group). The decision to use this number of cows has been validated by using the chi square test. At the time of presentation, the experiment will be well advanced with several sets of results that will give an indication of the likely outcome. Early results that have been analysed are indicative of an improvement in conception rate when using the modified ovulation synchronisation program that incorporates a second prostaglandin injection.

Conclusions: This experiment is not yet complete and will involve about 3000 cows (n=1500 in each group). At the time of the presentation, the experiment will be well advanced with several sets of results that will give an indication of the likely outcome.

Reproduction: (Dairy)

P03-003-195

Association between udder health and the calving-to-first service and calving-to-conception intervals in Holstein and Normande dairy cows

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Objectives: The aim of this study was to determine the effect of the infection status of the udder in the first month of lactation on the calving-to-first service (CFSI) and the calving-to-conception (CCI) intervals in Holstein and Normande dairy cows under grazing systems.

Materials and Methods: An observational study was carried out in five dairy herds in Caldas, Colombia. A convenience sampling was used to select 208 cows, 151 were Holstein and 57 Normande, 165 cows were multiparous, and 43 were primiparous. Cows were on a grazing system plus supplementation with grains according to milk yield. Mineral mixes and water were available ad libitum. Composite milk samples were collected on a weekly basis, starting in the first wk after calving up to four wk after calving. One sample was used to determine the somatic cell count (SCC), while the other one was used for culture only if SCC was above 100.000 cells/mL in primiparous cows, and 200.000 cells/ml in multiparous cows. An intramammary infection (IMI) was declared when the SCC arithmetic mean in the four wk after calving was higher than the cut-offs, and at least one culture was positive for a mastitis-causing pathogen. Information on the intervals from calving to first service and calving to conception was recorded.

The association between an IMI and the reproductive parameters was graphically evaluated using Kaplan-Meier survival graphs. Survival curves were compared using a Wilcoxon, Tarone-Ware, and Peto-Peto-Prentice tests. Further, parametric analysis were used to evaluate the fixed effects of herd, breed, parity, and milk yield. Analyses were performed using the commands `stset` and `streg` of Stata[®] 14.0 (StataCorp, College Station, TX, USA).

Results: The breeding period started 40 d after calving, as all herds followed a voluntary waiting period before the first service. Cows were followed up for 300 d after calving, any information about the failure of interest (conception) beyond this period was considered censored. According to the Kaplan-Meier survival graphs, the median survival time for CFSI was 75 d no differences were found according to IMI (P > 0.05). The survival graph for CCI showed a lower risk of conception in cows IMI (P < 0.05) Parametric models, exponential and Weibull, showed no effect of independent variables: breed, parity, average milk yield, and the interaction between IMI around calving and time (P > 0.05). Subsequently, the single effect of IMIs and time was evaluated. Both parametric models showed a significant effect of IMI in the first month after calving on the risk of conception in the follow-up period. At any time after calving, cows with IMI had 0.67 [=exp(-0.403)] times lower

risk of conception compared with negative cows. The predicted median from CC interval was 111 d and 135 d for cows without and with IMI, respectively. The shape parameter (p) of the Weibull time distribution was greater than one, indicating that the hazard of conception in both groups of cows was not constant and increased with time after calving.

Conclusions: The presence of IMIs in the first month after calving was associated with longer intervals from calving-to-conception; therefore, a cow will need a larger number of days become pregnant. Although cow-level factors did not affect the indices studied, there was an effect of the overall herd management that should not be dismissed as a potential explanation for the results

Reproduction: (Dairy)

P03-003-197

Key factors influencing reproductive performance in seasonal-calving dairy herds

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Objectives: Economic sustainability of seasonal calving dairy herds is dependent on fertility performance. In Ireland, our reliance on grass-based management systems, dictates that the vast proportion of dairy cows must calve within a pre-defined calving season to maximize economic output, to optimise milk production from grass, and crucially maximise postpartum periods for re-breeding. Breed, age, milk production, energy balance, and inter-current disease have all been demonstrated to influence dairy cow fertility. The aim was to evaluate the relative importance of herd and management factors on reproductive performance in seasonal calving dairy herds.

Materials and Methods: Breeding data were collected from 93 seasonal-calving Irish dairy farms to determine the relative importance of herd and management factors on reproductive performance, described in terms of two binary outcome variables: achieving (or not) a 65 % six-week pregnancy rate and a 15 % end of season not pregnant (empty) rate. Individual cow data were collapsed to attain herd level data pertaining to herd size, age, economic breeding indices, oestrous detection efficiency and accuracy, and calving pattern. Separate models were developed for each outcome variable, using backward-stepwise multivariable logistic regression analysis. The fit of the statistical models was evaluated using the Hosmer and Lemeshow goodness-of-fit test.

Results: The mean (\pm SEM) 24, 48 and 72 day submission rates were 73 (\pm 2.2), 87 (\pm 2.1) and 93 (\pm 1.8) %. Mean calving to conception interval was 97 days varying from 80 to 174 days for individual farms; suggestive that the predicted calving to calving interval was 377 days (range 362 to 414 days). The six-week and twelve-week pregnancy rates were 54 (\pm 1.7) and 72 (\pm 1.7) %, respectively. Concerning the six-week pregnancy rate: herds classified as having a lowered proportion (mean 44 %) of early calving cows were 28 (confidence interval; CI; 4.5 to 177) times less likely ($P = 0.003$) to achieve a high six-week pregnancy rate (> 65 %) compared with herds classified as having a high proportion of early calving cows (mean 58 % cows classified as early calvers). Herds with lowered oestrous detection efficiency were 6 (CI 1.2 to 28) times less likely to achieve a high (> 65 %) pregnancy rate compared with herds categorised as having good oestrous detection efficiency. Concerning

the empty rate: the mean empty rate was 19 % (range 0 to 41 %) for all farms. Herd size was the single factor associated with empty rate ($P < 0.01$). Only 13 and 16 % of medium and large herds, respectively, achieved an empty rate of less than 15 %, compared with 46 % in the smaller herds.

Conclusions: The existing calving pattern and oestrous detection efficiency were the greatest determinants of fertility in seasonal-calving herds. Management strategies aimed at improving oestrous detection and the calving pattern are essential and strategies must be employed to deal with late calving cows. Management of replacements to ensure early calving is key. The high empty rate is of concern as it severely limits a farm's ability to cull animals for reasons other than fertility. Larger herds were more at risk of not achieving high overall pregnancy rate. Greater attention is required in expanding herds to ensure fertility is not compromised.

Reproduction: (Dairy)

P03-003-198

Efficacy of nonsteroidal antiinflammatory drugs for the treatment of acute puerperal metritis in dairy cows

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Objectives: Acute puerperal metritis (APM) is a potentially life threatening, painful disease and is often treated with third-generation cephalosporins. An increasing antibiotic resistance is well documented and associated with decreasing clinical efficacy, animal welfare and economic consequences. Hence, there is the need to evaluate alternative therapies to antibiotics. Nonsteroidal anti-inflammatory drugs are frequently used as supportive treatment in addition to antibiotics and high self cure rates of APM are described. Therefore, the objective of this study was to compare the efficacies of ketoprofen and ceftiofur for the treatment of APM.

Materials and Methods: Between June 2013 and February 2015 a total of 610 dairy cows from 6 farms in Germany were enrolled. Inclusion criteria was a rectal temperature (RT) ≥ 39.5 °C and a reddish-brown fetid vaginal discharge within the first 10 DIM. Cows meeting the inclusion criteria were randomly allocated to treatment with ketoprofen (3 mg/kg of body weight, $n = 300$) or treatment with ceftiofur (1 mg/kg of body weight, $n = 310$), both administered on 3 consecutive days. Rectal temperature was recorded daily for a period of 7 days after enrollment. Cows that showed RT ≥ 39.5 °C on day 4 to 7 after inclusion received an extended treatment (extT) with ceftiofur for 3 (ketoprofen group) or 2 (ceftiofur group) more days. Between 21 and 40 DIM cows were examined with the metricheck device and vaginal discharge was categorized on a 5-point scale according to the presence of pus.

Results: A total of 52 cows (35 from ketoprofen group, 17 from ceftiofur group) were excluded from analysis due to missing protocol compliance ($n = 37$) or concurrent disease ($n = 15$). Cows initially treated with ketoprofen were more likely to have an extT than cows treated with ceftiofur (61% vs 31%, odds ratio 3.47, $P < 0.01$, $n = 558$). Occurrence of purulent vaginal discharge (PVD) was similar for both treatment groups (ketoprofen: 56%, ceftiofur: 53%, $P = 0.64$). However, cows with an extT had 2.12 ($P < 0.01$, $n = 438$) times the odds of PVD than cows without extended treatment. Treatment group did not affect mean

100 DIM milk yield ($P = 0.58$), first artificial insemination (AI) pregnancy risk ($P = 0.25$), time to first AI ($P = 0.65$) and time to pregnancy ($P = 0.56$).

Conclusions: More than half of the cows initially treated with ketoprofen needed an extT with ceftiofur. However, the number of ceftiofur doses administered could almost be cut in half as well without negative effects on the prevalence of PVD, milk yield, and reproductive performance.

Reproduction: (Dairy)

P03-003-199

Effect of puerperal uterine diseases on histopathological findings and mRNA expression of pro-inflammatory cytokines of the endometrium in dairy cows

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Objectives: The aim of this study was to examine if uterine diseases occurring during the first two weeks after parturition have a long-lasting negative effect on histopathological and molecular parameter of the endometrium in dairy cows.

Materials and Methods: Forty-nine lactating Holstein Friesian cows were divided into two groups, one without (UD-; $n=29$) and one with uterine diseases (UD+; $n=21$), defined as retained fetal membranes and/or metritis. General clinical examination, vaginoscopy, transrectal palpation and transrectal B-Mode sonography were conducted on Days 8, 11, 18, 25 and then every 10 days until Day 65 (Day 0=day of calving). The first endometrial sampling (ES1; swab and biopsy) was done during estrus around Day 42 and the second ES (ES2) during the estrus after synchronisation (cloprostenol between Days 55 and 60 and GnRH 2 days later). Swabs were used for aerobic bacterial examination. Endometrial biopsy samples were evaluated histopathologically; inflammation was categorized as acute purulent, chronic purulent and chronic non purulent endometritis. Gene expression of pro-inflammatory cytokine (interleukin (IL)1 α , IL1 β , IL6 and tumor necrosis factor (TNF) α) in the endometrium were determined by quantitative reverse transcriptase PCR.

Results: Cows of groups UD- and UD+ showed no differences ($P>0.05$) in the frequency of histopathological inflammatory alterations of the endometrium and in the prevalence of positive bacteriological cultures. However, UD+ cows more often had a chronic purulent endometritis (ES1; $P=0.07$) and an angiosclerosis (ES2; $P\leq 0.05$) than UD- cows. Endometrial gene expression of pro-inflammatory cytokine IL1 α (ES2), IL1 β (ES2) and TNF α (ES1 and ES2) were higher ($P\leq 0.05$) in cows of the UD+ group than in the UD- group.

Conclusions: In conclusion, puerperal uterine diseases in cows affect the endometrium not only during the puerperium, but also during the postpuerperal period by inducing histopathological alterations as well as a rise in gene expression of pro-inflammatory cytokines.

Reproduction: (Dairy)

P03-003-200

Dynamics of pregnancy loss in Jersey, Holstein, and Jersey x Holstein crossbred cows in large multibreed dairy herds

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Objectives: The objective was to analyze the dynamics of pregnancy losses (PL) for the genetic groups Jerseys (JE), Holsteins (HO), and Jersey x Holstein crossbreds (JH), considering parity, disease occurrence, year and season in large multibreed dairy herds in Texas, USA.

Materials and Methods: The study analyzed lactation records from 3 dairy herds located in NW Texas, USA. Herds included Holstein, Jersey, and Holstein x Jersey crossbreds. A total of 25,840 breeding records of cows calving between January 2005 and December 2011 were available. Data were extracted from on-farm software and consisted of cow identification number, breed, calving date, parity, date of breeding and sire, pregnancy check date and outcome, herd code, and recorded health disorders and date. Early pregnancy diagnosis (around 35d) was performed by one of the authors (farm veterinarian) by transrectal ultrasonography and pregnancy reconfirmation was done by palpation per rectum. A diagnosis of pregnancy loss was made when a cow that was determined pregnant was subsequently diagnosed as non-pregnant or received a subsequent breeding. Data were analyzed by logistic regression and explanatory variables included genetic group, disease occurrence, parity, year, season, and herd.

Results: Pregnancy loss for the overall period was 8.9%. Percentages of pregnancy loss per year were 16.8,

12.8, 15.8, 10.9, 6.3, 9.1, and 4.2 for 2005, 2006, 2007, 2008, 2009, 2010, and 2011, respectively. Regarding genetic group, values for PL were 10.1, 7.3, and 4.9 for HO, JE, and JH, respectively. The odds of PL were significantly higher for HO ([95%CI] = 2.16 [1.62-2.86]) and JE ([95%CI] = 2.07 [1.27-3.36]) cows compared with JH crossbred. Cows that had a disease event during lactation had a PL of 11.2% compared to 8.4% in healthy cows. The odds of PL were significantly higher for cows with at least one disease event ([95%CI] = 1.34 [1.20-1.48]) compared with healthy cows. Pregnancy loss was lowest for breedings in fall (7.77%) and highest for breedings in spring (10.2%).

Conclusions: The level of pregnancy loss in this population of dairy cattle decreased during the study period, was associated with occurrence of disease, and was significantly lower in the JH crossbred than in the pure breeds.

Reproduction: (Dairy)

P03-003-201

Randomized clinical trial of intrauterine cephalosporin in dairy cows for the treatment of purulent vaginal discharge and cytological endometritis

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Objectives: Purulent vaginal discharge (PVD) and cytological endometritis (ENDO) diagnosed by cytobrush are associated with detrimental impact of reproductive performance of dairy cows. Research suggested that ENDO could be diagnosed using a leukocyte esterase colorimetric test (EST). The efficacy of intrauterine cephalosporin for treating PVD is known but its efficacy for treating ENDO diagnosed by cytobrush or by EST remains unclear. The objectives of this study were to validate ENDO diagnostic approaches (cytobrush and EST), and to quantify the impact of cephalosporin on the reproduction at first service of postpartum dairy cows affected by these diseases.

Materials and Methods: A total of 2259 Holstein cows from 28 commercial dairy herds were enrolled a randomized clinical trial. At 35 (\pm 7) days in milk (DIM), cows were diagnosed for PVD (purulent vaginal discharge or worse) and ENDO (\geq 6% polymorphonuclear cells using the cytobrush technique or at least small amounts of leukocytes using the EST). Regardless of reproductive tract disease status, cows were randomly assigned to receive an intrauterine cephalosporin infusion or no treatment. Statistical analyses were conducted using multivariable mixed logistic regression models adjusted for confounders and herd clustering effect.

Results: Prevalences of PVD, ENDO (cytobrush) and ENDO (EST) were 19, 35, and 49 %, respectively. Intrauterine cephalosporin treatment was associated with an increased first service pregnancy risk in cows diagnosed with PVD (no treatment: 15.4%; treatment: 31.4%) and ENDO (cytobrush: no treatment: 16.2%, treatment: 24.4%; EST: no treatment: 15.8%; treatment: 25.1%), but not in cows unaffected by any form of reproductive tract disease (no treatment: 34.8%; treatment: 32.6%). Kappa value (agreement) between the cytobrush technique and EST was 0.43.

Conclusions: Overall, an intrauterine infusion of cephalosporin improved first service pregnancy risk in cows with PVD and ENDO. Although, the cytobrush and EST diagnostic approaches did not provide the same information for ENDO diagnosis, they were both associated with a positive benefit on first service pregnancy risk when cows were treated with intrauterine cephalosporin.

Reproduction: (Dairy)

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Investigations of mammary and uterine blood flow in relation to milk yield, postpartum disease and pregnancy result in dairy cows

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Objectives: Milk yield of dairy cows has increased continuously during the last few decades while fertility parameters such as conception rate have declined. The objective of this study was the determination of blood flow variables of dairy cows in the uterine arteries and the pudendoepigastric trunks, which supply the mammary gland. These variables were related to the occurrence of uterine diseases, milk yield, and pregnancy result.

Materials and Methods: To achieve this, 119 multiparous German Holstein cows were examined transrectally using color Doppler sonography once during the dry period before parturition and on Days 7, 14, 28, 42, 56, 66, 76, 86 and 96 postpartum (pp). Milk yield and postpartum diseases were documented. After day 43, cows were inseminated during spontaneous estrous cycles or they underwent an OvSynch protocol and fixed-time AI. Cows that were not bred by day 56 were examined ultrasonographically every 10 d. Cows were examined ultrasonographically for pregnancy 26 d after AI, and in pregnant cows the vitality of the embryo was confirmed on day 42. Examinations were discontinued on day 42 when a cow was confirmed pregnant or on day 150 in cows that failed to conceive.

Results: Mean daily milk yield of all cows for the first 42 days was 37.7 ± 6.2 kg (mean \pm SD; min. 15.2, max 52.4 kg). Milk yield was correlated with BFV in the pudendoepigastric trunks on examination Days 7 to 96 pp with the exception of Day 76 ($P < 0.05$), and with TAMV on Days 7 and 14 pp ($P < 0.05$). The PI was greater in the left trunk ($P < 0.05$).

Depending on the mean daily milk yield in the first 42 days pp, 57 cows classified as high-milk-yielding (HMY) having a mean daily production of 42.7 ± 2.7 kg, and 62 cows classified as low-milk-yielding (LMY) had a mean production of 33.1 ± 4.8 kg.

On day 150 pp, 75 of 119 cows (63 %) were pregnant. Pregnancy result did not differ between LMY (43 of 62 pregnant, 69%) and HMY cows (32 of 57 pregnant, 56%; $P > 0.05$).

Mammary blood flow volume (BFV) was greater in pregnant HMY cows than in pregnant LMY cows on days 7, 14, 28 and 42 ($P < 0.05$), but time-averaged maximum velocity (TAMV) and pulsatility index (PI) did not differ between the two groups. Mammary BFV did not differ between open HMY and LMY cows ($P > 0.05$). Uterine blood flow did not differ between HMY and LMY cows ($P > 0.05$). Mammary and uterine blood flow did not differ between pregnant and open HMY cows and between pregnant and open LMY cows ($P > 0.05$). Likewise, mammary and uterine blood flow did not differ between pregnant and open cows of both production levels combined ($P > 0.05$).

Cows with retained fetal membranes (RFM) or metritis had a higher BFV and TAMV and a lower PI in the uterine arteries on Days 7, 14 and 28 pp ($P < 0.05$).

Conclusions: Milk yield did not affect pregnancy result and was not related to uterine perfusion. Increased uterine perfusion in cows with RFM and metritis may be due to increased uterine size attributable to delayed involution. High mammary perfusion in high-yielding cows is due to an increased demand for nutrients and oxygen. Color Doppler sonography is useful for the investigation of uterine and mammary perfusion in the context of puerperal disease and milk yield.

Reproduction: (Dairy)

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The effect of hypocalcemia and two different calcium treatment forms on uterine contractility in early postpartum cows

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Objectives: Aged lactating cows are exposed to hypocalcemia around calving, which may cause a declined smooth muscle activity of the myometrium. This may contribute to the reduced expulsion of the uterine content, thereby negatively influencing the process of involution. Various calcium treatment forms are used in dairy practice, but the exact effects of such products on puerperal uterine contractility are not exactly known. The aim of this study was to determine the effect of subclinical hypocalcemia and two forms (intravenous and per os) of calcium treatment on uterine contractility early postpartum.

Materials and Methods: Myometrial contractions of normocalcemic (n=14; blood Ca²⁺>1.06 mmol/l) and hypocalcemic cows (n=38; blood Ca²⁺<1.06 mmol/l) at a large-scale dairy cattle farm were examined using an open tip catheter system, that is suitable for measuring intrauterine pressure (IUP) changes. The first recording started 14-17 hours after calving and further three observations were performed in 12 hours intervals from the beginning of the previous session. Hypocalcemic cows were randomly treated either with intravenous (n=10; Cofacalcium inj. A.U.V., CoopHavet), or oral (n=10; ReCovin Calcium paste, Kruuse) calcium 1 hour after the beginning of the first recording once. Data of intrauterine pressure changes were collected from the previously gravid uterine horn, and after amplification and digitalisation they were analyzed using a semiautomatic, operator-made software (LabVIEW 5.0, National Instruments). Contraction frequency, amplitude, duration, mean and total area under the pressure curves were calculated. Coccygeal blood was withdrawn at the beginning of the first and at the end of all recordings and Ca²⁺ was measured using a portable blood gas and electrolyte analyzer on site (ABL-77, Radiometer). Correlation analysis and two-sample t-tests were used for statistical analysis.

Results: Positive correlation was found between blood Ca²⁺ concentration and the frequency of myometrial contractions 14-17 hours postpartum (r=0.61; P<0.001). Cows with retained fetal membranes (n=14) had significantly higher frequencies than non-retained cows (P<0.001). Cows treated with a single dose of oral calcium did not show any significant differences as related to the untreated control cows in terms of uterine contractility during the first measurement, but their mean contraction frequency was significantly (P<0.05) higher during the second. Six cows that received intravenous calcium showed a marked increase (238%) in contractility during the first hour after the infusion. However, in 4 cows such an increase could not be observed.

Conclusions: Subclinical hypocalcaemia had a negative effect on uterine contractility. In most cases intravenous calcium treatment was very effective to improve myometrial activity. Oral calcium supplementation did not cause such a marked elevation of uterine mechanical activity, but it might have a possible beneficial long-term effect.

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Reproduction: (Dairy)

P03-003-204

The relationship between fatty acid profiles in milk identified by fourier transform infrared spectroscopy and onset of luteal activity in dairy cows

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Objectives: Milk components represent information on the metabolic state of the milking cow. Previous work has shown milk fat:protein ratios in milk to be unreliable predictors of reproductive performance (1) and direct measures of progesterone are expensive. Fourier transform infrared spectroscopy (FTIR) has previously been used for online measurement of milk components in the wet phase (2). Drying the milk sample before analysis decreases component detection limits and improves estimate accuracy (3). This study aimed to use dry phase to measure different components of the milk fat fraction and to compare these to onset of luteal activity (OLA).

Materials and Methods: Morning milk samples were collected thrice weekly (Monday, Wednesday, Friday) for the first 10 weeks in milk (WIM) from 87 lactations in 73 healthy Norwegian Red cows over two winter housing seasons. These samples had bronopol added to them before being frozen, thawed, and analysed by enzyme linked immunoassay to determine progesterone concentration and the proportions of the milk fatty acids which were C4:0, C14:0, C16:0, C18:0, and C18:1cis9 using dry film FTIR (3), and averaged by WIM. OLA was defined as the first day that milk progesterone concentrations were >3ng/ml for two successive measurements, the study population was categorised as early (n=47) or late (n=40) OLA, using the median value of 21 DIM as the cut off. Further milk samples were collected 6 times weekly, from morning and afternoon milkings, these were pooled by WIM and one proportional sample was analysed fresh for fat, protein and lactose content, using infrared spectrography. Feed intakes, bodyweight, milk content and yield were recorded. These data were used to calculate daily energy balance (EB) in 42 lactations before they were averaged by WIM at the cow level. Relationships were explored for each WIM using the student's t test (EB), or logistic regression (Early OLA vs. late OLA).

Results: Higher proportions of medium chained fatty acids (C14:0 and C16:0) were found in the early, than in the late OLA group from WIM 1 until WIM 7 (weekly p value always <0.05). In WIM 4, a lower milk fatty acid proportion of C16:0 predicted late OLA with 74% sensitivity and 80% specificity. The proportion fatty acids that were long chained (C18:0 and C18:1cis9) was consistently lower in the milk of the early OLA group and the difference was greatest in WIM 4 and 5. A higher proportion of C18:1cis9 was seen in the milk fatty acids of the late OLA compared to the early OLA group from WIM 1 until WIM 5 (all weekly p-values <0,05). Animals experiencing late OLA had a more negative EB in WIM 1, 3, 4, and 5 (p-values < 0.05) than those in the early group. The greatest differences were in WIM 3 and 4. No relationship was seen between OLA and the content of either protein or fat in milk, or between OLA and the milk fat:protein ratio. The differences in milk fatty acid concentrations between the two groups were most likely the result of differences in energy balance between the two groups (4).

Conclusions: This study showed that OLA is related to specific milk fatty acid concentrations. The relationships occur from WIM 1. When OLA is dichotomised as early or late, univariate analysis found prediction accuracy to be highest in WIM 4. This study also showed that dry film FTIR performed on previously frozen milk samples can determine milk fatty acid composition.

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Reproduction: (Dairy)

P03-003-205

The effects of building design on hazard of first service in Norwegian dairy cows

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Objectives: Legislation passed in Norway bans the use of cattle tie stalls from 2024. Dairy producers with these facilities must build cubicle sheds or cease cattle production by 2024. Therefore, there is a requirement for information about how building design influences cattle health and production so that building designs can be optimized and returns from capital investments maximized. This study aims to investigate the effect of specific environmental factors such as herd size, stocking density, floor type, and building layout on calving to first service in Norwegian dairy cattle.

Materials and Methods: This study was part of a larger descriptive and cross-sectional project on freestall housing in Norwegian dairy herds. Inclusion criteria for the study were: farmers willingness to participate, herd size > 20 standardized cow years (based on the year 2005), barns built between 1995 and 2005, and the presence of free stalls. All 232 farms that fulfilled the inclusion criteria were included in the study. The herds were visited once during the indoor housing period between September 2006 and June 2007 by trained technicians. The technicians recorded: type of flooring (in the alleys and feeding area), Free accessible area (alleyways, freestalls, feeding areas), number of alleyways, number of dead-ending alleyways, and the total number of freestalls present on the farm for the adult cows. Herd level data were gathered from NDHRS database these included average herd milk yield per cow year (total milk delivered from the farm annually / cow years), and herd size (in cow years). Lactation level data were also extracted from this database. The final dataset contained data on 38,436 calvings and 27,127 services. Time to event data were analyzed using Cox proportional hazard modeling techniques. The explanatory variables were tested in univariate cox-proportional hazard models, a frailty term for the variable farm was included in the model. A crude hazard ratio for each explanatory variable was generated. Explanatory variables with a univariate P-value of <0.10 were included in a forward stepwise procedure to build a multivariable model.

Results: Univariate cox proportional hazard analyses showed that herd size and milk yield were positively associated with HFS. Total free accessible area (FAA) and FAA available per cow year were positively associated with the HFS, as was the number of freestalls available per cow. Cows housed on slatted floors had a lower HFS than those housed on solid floors. Conversely, cows housed on rubber floors had a higher HFS than cows on concrete floors. Dead-ending alleyways reduced the hazard of AI after calving. A multivariable cox proportional hazards model, accounting for herd management by including a frailty term for herd, showed relationships between hazard of postpartum service and explanatory variables. Animals in herds with more than 50 cows had a higher HFS (Hazard ratio (HR) 3.0) compared to those in smaller herds. The HFS was also higher (HR 4.3) if there was more than 8.8 M² of space available per cow year compared to herds in which animals had less space. HFS after calving increased with parity (parity 2 HR 0.5, parity ≥ 3 HR 1.7), and was reduced if a lactation began with dystocia (HR 0.82) or was a breed other than Norwegian Red (HR 0.2). The frailty term, herd, was large and highly significant indicating a significant proportion of the variation resides at herd level. The hazard of first insemination decreased with time for all predictive variables, except dystocia.

Conclusions: This study shows that providing adequate environmental conditions for estrus behavior is imperative for reproductive efficiency, also after herd management factors and time from calving have been accounted for. Thus optimizing building design for reproductive efficiency is of significant importance when constructing new cattle housing.

Reproduction: (Dairy)

P03-003-207

Comparative metagenomics of the bovine endometritic and healthy uterine microbiota and its predicted functional diversity by shotgun sequencing

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Objectives: The aim of this study was to characterize by the first time, using metagenomics shotgun DNA sequencing, the intrauterine microbial population and its predicted functional diversity from healthy cows and cows affected with endometritis. We expected to identify relationships of certain microbial genes with postpartum uterine health.

Materials and Methods: In total, 20 Holstein dairy cows at 25-35 days postpartum from a single farm located near Ithaca, NY, were enrolled in the study. Enrolling period was from June 7th, 2014, to June 14th, 2014. Endometritis diagnosis was diagnosed by retrieving and scoring vaginal discharge using the Metrichick[®] device. Intrauterine samples for microbiological analysis were taken by cytobrush technique from the 20 cows, 8 cows diagnosed with endometritis and 12 healthy cows. After sampling, the cytobrushes were transferred to a DNA/RNA free 2 ml microcentrifuge tube containing 1.5 mL of DNA/RNA free water. Following vortex, cytobrushes were removed from tubes and the suspension was centrifuged. Pellet was used for extraction of total DNA using PowerSoil DNA Isolation Kit. After normalization the samples were used as an input to the Nextera XT DNA Sample Prep Kit. Pair-end sequencing was performed using the MiSeq Reagent Kit v3 through the Illumina MiSeq platform. Additionally, total number of bacterial 16S rDNA copies in uterine samples was detected by Real-Time PCR.

Sequences were uploaded to the MG-RAST pipeline to determine relative abundance of the microbiota and to predict functional abundance. The non-redundant multi-source protein annotation database (M5NR) and SEED were the annotation source used.

Results: Uterine samples sequences were assigned to Bacteroidetes, Proteobacteria, Firmicutes, Fusobacteria, Actinobacteria, and Spirochaetes. Fusobacteria was almost absent in healthy uterine microbiota, representing about 0.5% of the population. The most abundant genera within Fusobacteria phylum (Propionigenium, Ilyobacter and Fusobacterium) were significantly more abundant in endometritic cows. Genus Trueperella was absent in healthy uteri. E. coli were the most abundant species in both microbiotas, but did not show statistical difference. Resistance to antibiotic and toxic compounds (RATC) was the predominant subsystem identified in the virulence, disease, and defense system (greater than 50%) for both microbiotas. Multidrug resistance efflux pumps were the highest category identified in RATC subsystem, followed by resistance to fluoroquinolones, copper homeostasis and cobalt-zinc-cadmium resistance. Toxins were unique to endometritic microbiota and Cytolethal distending toxins were predominant. Antibacterial peptides were restrictedly found in healthy microbiota and dominated by tolerance to colicin E2. Capsular and extracellular polysaccharides genes, LPS assembly and Lipid A biosynthesis were higher in healthy microbiota. When Lipid A modification was predicted, it was present only in endometritic samples. Bacterial responses to DNA damage and environment stress were more abundant in healthy microbiota. No difference was observed in total bacterial load between microbiotas.

Conclusions: The study provides a deep insight into uterine microbial community and its dynamics. Bacteria in endometritic and healthy uteri did not show abrupt variations. A microbial core is determinant to define health and disease. Species intrinsic factors may be more relevant to the development of disease than abundance. The prediction of the functional diversity of endometritic and healthy cows may serve to identify unknown etiological agents and virulence factors that contribute to disease, grant therapy development and prevention. Our findings suggest that the endometritic microbiome harbors unique functions that deserve further investigation.

Reproduction: (Dairy)

P03-003-208

Comparison Of Ovsynch And Trans-Rectal Palpation As A Treatment For Anestrus In Israeli Dairy Farms

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Objectives: Dairy farms not applying timed artificial insemination reproduction programs rely on estrus detection for managing herd reproduction. As dairy producer's numbers are declining while the average farm size grows worldwide, automated estrus detection systems are becoming more available. This fact, combined with growing interest in reproductive programs with minimal pharmacological intervention due to public concerns about food safety, present a need for an efficient focused anestrus treatments. The purpose of the presented study is to compare the efficacy of OVSYNCH and ovarian palpation as a treatment for anestrus field cases.

Materials and Methods: The presented study is a multi-centered, randomized, controlled trial designed as a prospective cohort study. The

study was conducted on lactating cows that were submitted for anestrus examination between May 2010 and May 2011 in four commercial Israeli dairy herds consisting of 250 to 600 Israeli Holstein cows.

Control cows not detected in estrus were examined by the attending veterinarian using Trans-rectal palpation and were treated according to the findings on the ovaries. If a corpus luteum (CL) larger than 20 mm in diameter was diagnosed, the cow was treated with a PGF_{2α} analog; 500 µg Cloprostenol Sodium. If a CL was not diagnosed in two trans rectal palpations week apart, the cow was regarded as acyclic, and was treated by IM injection of GnRH; 200 mg Gonadorelin Acetate. Cows treated with GnRH were re-examined 2 weeks later for the presence of a CL. If an acyclic cow did not develop a CL 3 weeks after GnRH treatment, it was treated with IM injection of 5000IU hCG or with a progesterone intravaginal insert. All cows were artificially inseminated at electronically or visually detected estrus.

Cows allocated to the OVSYNCH treatment group were not examined; instead the OVSYNCH protocol was initiated. OVSYNCH cows that were detected on estrus before the completion of the protocol were submitted for artificial insemination by the AI technician. If not detected in estrus, OVSYNCH cows were AI by the designated date of the OVSYNCH protocol.

Results: 488 cows were included in the study; 299 (61.3%) OVSYNCH and 189 (38.7%) Control. OVSYNCH and control groups differed in only in the proportion of cows calving in the summer (P<0.001). OVSYNCH cows had shorter waiting period compared with control cows (median time to 1st AI 104 and 111 days respectively, P=0.003). The OS cows were inseminated closer to the submission for anestrus examination in comparison to the control group (average time to AI 14 and 18.6 days respectively; P=0.043). At submission to AI, higher proportion of the Control cows were not inseminated, as the AI technician did not find physiological estrus signs (29.6% and 17.7% for Control and OS cows respectively; P=0.002).

Conception rates in July and August differed from the other year months. The study groups did not differ neither in crude 1st AI conception rate (25.4% and 28.4% respectively, P= 0.464), nor when controlling for summer inseminations in the multivariate analysis (OR=1.164, P=0.478). The pregnancy rate in the study groups did not differ at 150 DIM, but tended to be higher for OVSYNCH cows at 200 DIM (OR to be empty =0.721, P=0.085).

The time to conception was analyzed using survival analysis for 3 different time periods: Days empty, 1st AI to conception interval and anestrus examination to conception interval (Effective waste days).

The OS group tended to conceive earlier in the lactation in comparison to the palpated group (P=0.082), but no significant differences were demonstrated between the treatment groups in 1st AI to conception interval and for effective waste days.

Conclusions: Anestrus treatment by the application of the OVSYNCH protocol as a part of anestrus management can be effective substitute of traditional ovarian palpation. In the last decades the emphasis of reproduction management is the herd, not the single cow. The economic efficacy of the implementation of individual treatment of anestrus is questioned. However, the public concerns about food safety and animal husbandry ethics may lead to diminishing consumer acceptance of food manufactured by systematically hormone treated animals.

Reproduction: (Dairy)

P03-003-209

Conception is all the more reduced that mastitis and subclinical ketosis simultaneously occur around artificial insemination

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Objectives: Reproductive performances of dairy cows are recognized as a key parameter for profitability of farms, but they are getting worse continuously in many countries. Many reasons of these changes were proposed, and among others, the health status of cows. Mammary infection and subclinical ketosis (SCK) have been reported to have deleterious impact on conception, but their interactions and dynamics are not fully understood. The objective of this study was to better describe the relationship between reproductive performances and udder health, accounting for the interaction with SCK.

Materials and Methods: Data from the national French dairy milk improvement system and data on the artificial inseminations (AI) from 2007 to 2012 were included. Conception at first or at all AI was explained by (i) the udder health status and (ii) the presence of SCK on the test-days around AI. To define the udder health around AI, somatic cell counts (SCC) were classified as High or Low before and after AI, and transformed into four categories (LL, and LH, HH, HL) relative to various thresholds (from 200,000 to 400,000 cells/mL). SCK around AI was defined thanks to fat and protein contents or to their ratio before or after AI. Among the definitions of SCK there were: (i) fat before AI >45 g/L and protein between 35 and 45g/L, (ii) fat : protein ratio before or after AI >1.5, (iii) protein before AI <30 g/L, (iv) protein before AI <30 g/L and protein after AI <28 g/L.

A mixed binomial model adjusted by the lactation stage, milk yield and parity was applied yearly on the whole population or on subpopulations with or without SCK. Herd was kept as random effect in all models.

Results: The relative risk (RR) of conception were 0.86 (95%CI = 0.84-0.87), 0.98 (95%CI = 0.96-1.00) and 0.85 (95%CI = 0.84-0.86) for LH, HL and HH compared to LL, respectively. The same results were observed for the first AI or all AI, and whatever the SCC threshold used. RR of conception was 0.87 (95%CI = 0.84-0.90) with SCK compared to without. Depending on the SCK definition, the RR varied from 0.86 (95%CI = 0.83-0.89) to 0.90 (95%CI = 0.89-0.91). Altogether, this shows that (i) an increase in SCC around AI or (ii) presence of SCK around AI were both associated with a decrease in the conception rate.

The same results are observed in the subpopulation without SCK. The relationships between SCK and conception on one hand and SCC change and conception on the other hand were not independent. First, when performed on the subpopulation with SCK only, the RR of conception for LH compared to LL cows is decreased to 0.74 (95%CI = 0.84-0.87) instead of 0.86 for the whole population. Second, including the interaction term SCK * class of SCC for the regression on the whole population showed that the reduced risk of conception for LH compared to LL was two times higher in the presence of SCK compared to without SCK. Results were robust, even if minor changes in coefficients were observed between the various years of study.

Conclusions: The present results showed that conception success was reduced by around 15% for cows with high SCC or with an increase of SCC around AI. This association was strengthened by including an indicator of SCK and when models were run only on cows with SCK, showing that the relationship between udder health and conception could

be modulated by metabolic disorders such as negative energy balance. These results are of great interest for herd management. Moreover, they support the idea that a local inflammatory reaction may affect systemic functioning and interact with the physiology of distant organs.

Reproduction: (Dairy)

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Sensitivity of 21-day Pregnancy Risk to Stated Voluntary Wait Period: A Simulation Study

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Objectives: 21-d Pregnancy Risk (21-d PR) is used to monitor reproduction in dairy herds with non-seasonal calving patterns. It indicates the percentage of eligible cows which become pregnant in a 21 day period. The calculation requires that a voluntary wait period (VWP) be specified although the standardised version uses a 50-day VWP. A common trend in certain regions is to delay the VWP and use universal synchronisation of first insemination, which achieves both high conception risks to first insemination and exceptional VWP-adjusted 21-d PR. The objective of this study was to investigate the sensitivity of 21-d PR to the VWP specified in calculations.

Materials and Methods: A daily Markov-chain model was designed to simulate fertility in an individual cow and her replacements. This model required three prior probabilities for conception risk; at first timed insemination (1st TAI), to oestrus detection, and to resynchronisation. It also required two inputs for 21-d Oestrus Detection Risk, defined as the percentage of eligible cows which were detected in oestrus in a 21 day period; one for each of the periods before and after 1st TAI. Prior probabilities were formulated after literature review, informed by practical experience. The daily probability of oestrus detection was modified by the pattern of reinsemination from a large dataset. Probabilistic components of the model were repeatedly drawn from Bernoulli distributions using RiskAMP v4.76 (Structured Data LLC, USA). After a burn-in period of 365 days and assuming a uniform initial calving distribution, herd performance was simulated over three years. Back-door Ovsynch, Presynch-Ovsynch with and without oestrus detection before 1st TAI ('cherry-picking'), and Double Ovsynch were investigated. For all protocols, 1st TAI occurred at 76 days postpartum. Where applicable, oestrus detection before 1st TAI began at 50 days postpartum. Annual average 21-d PR were calculated using various specified VWP and eligibility criteria emulating DairyComp 305 (Valley Agricultural Software, USA). Multiple runs were performed with a simulated herd size of 250 to investigate the distribution of 21-d PR for each specified protocol, followed by runs of 10,000 cows to show the sensitivity of 21-d PR to VWP.

Results: 21-d PR had variable sensitivity to the VWP used in calculations, with both a greater numerical and proportional sensitivity either with universal synchronisation of first insemination, or with improved fertility management after first insemination. The difference was greatest where both factors combined, such as for a model of the Double Ovsynch protocol with 25d early resynch and excellent oestrus detection after 1st TAI, which achieved a long-run 33.7% (95% CI: 33.3-34.0%) 21-d PR when calculations used the 76 day VWP, or 24.3% (95% CI: 24.1-24.6%) when calculations used the standard VWP. In contrast, a similar model using Presynch-Ovsynch with cherry picking achieved a long-run 26.9% (95% CI: 26.6-27.2%) 21-d PR when calculations used the 76 day VWP and 24.9% (95% CI: 24.7-25.2%) with the standard VWP. Given that its denominator is 'all eligible cows', 21-d PR is effectively weighted by the

size of each eligible group. With improving 21-d PR, and particularly with universal synchronisation of the first insemination, weighting moves from a broadly linear decline with days postpartum to a broadly logarithmic decline. Distributions of eligible groups and standardised 21-d PR for each protocol will be displayed.

Conclusions: Given the variable sensitivity of 21-d PR to VWP, it should be made clear when calculations use an adjusted VWP. This is particularly important where universal synchronisation of first insemination is practiced, or where excellent management of reinseminations is achieved. The mathematical characteristics of 21-d PR place exponential emphasis on fertility in early lactation with incremental improvements in 21-d PR. Manipulation of VWP in calculations may hide potential for further improvements, depending on regional economics. Expected distributions of 21-d PR using a 50-d standardised VWP may be useful for troubleshooting herd management.

Reproduction: (Dairy)

P03-003-211

Using a single measurement of insulin-like growth factor-1 to predict the likelihood of reproductive tract health at day 45 postpartum in dairy cows

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Objectives: To determine the relevance of : i) vaginal mucus character score, ii) uterine ultrasound scanning (scan), iii) use of cytobrushing of the uterine body endometrium as methods to evaluate reproductive tract health, and iv) whether a single blood sample for IGF-1 concentration collected in week 3 postpartum (pp) can be used as a predictor of reproductive tract health on day 45 pp.

Materials and Methods: The trial involved a herd of 98 Holstein cows. Blood sampling to monitor IGF-1 concentration in week 3 pp., and weekly evaluation of reproductive tract health pp, using ultrasonography and vaginal mucus scoring, were carried out. Twice weekly milk sampling was used to monitor progesterone and resumption of ovulation pp. (Forde et al., 2011). Lameness was assessed every 2 weeks pp. (Sprecher et al., 1997). After day 21 pp., when a clear vaginal mucus sample and normal uterine scan were obtained on two consecutive weekly analyses a cytology brush sample from the uterine body endometrium was carried out (Kasimanickam et al., 2004). Reproductive tract health was graded on day 45 pp. Indices of health status were defined as: i) normal uterine scan (Mee et al., 2009), ii) vaginal mucus score of 0 (Williams et al., 2005), and iii) not lame (non-infectious causes were excluded). Health status was graded 0 to 3. A grade of 0 indicated that the normal threshold for all indices had been exceeded (cows were in poor health); grade 1 indicated that 1 health index was normal; grade 2 indicated that 2 health indices were normal; grade 3 indicated that all 3 health indices were normal. The more healthy indices cows had at day 45 postpartum the better their "health status" was at that time. Using this grading system, there were 3 possible outcomes that cows could achieve: i) full health (3 healthy indices); ii) 3 or 2 healthy indices rather than 1 or 0 healthy indices, and iii) 3, 2, or 1 healthy indices rather than 0 health index. AREC (UCD) granted ethical approval.

Results: In weeks 3 to 5, the percentage of mucus samples with scores ≥ 2 was 51, 45, and 43%, respectively; with a steady decline up to week 9 pp. Cytobrush samples (n = 56 cows) found subclinical endometritis

in 43% (24/56) of cows that had 2 consecutive, weekly, clear mucus scores. Only 47 of the 56 cows had a scan taken concurrently with the cytobrush. Thirty of these 47 cows had a normal uterine scan and yet subclinical endometritis was confirmed in 40% (12/30) of them. Odds of involution before or during any of weeks 3 to 5 pp was greater that week for a normal uterine scan (odds ratio; OR = 8.26). Probability of involution did not depend on the mucus score being 0 or > 0. Neither a clear mucus sample (score = 0) nor a normal uterine scan resulted in a greater probability of ovulation either before or during any of weeks 3 to 5 pp. Odds of a healthier reproductive tract on day 45 pp increased by 41% if a cow's IGF-I concentrations were 25 ng/mL higher than another cow in week 3 pp.

Conclusions: i) 2 consecutive clear mucus scores or normal scans do not infer absence of subclinical disease (diagnosed by uterine cytobrush); ii) odds of involution either before or during weeks 3 to 5 pp increased with a normal uterine scan that week but not with a clear mucus score; iii) probability of ovulation either before or during weeks 3 to 5 pp was not related to the uterine health markers (scan or mucus score); iv) a significant relationship was found between IGF-I concentrations in week 3 pp and the reproductive tract health status of cows at day 45 pp. This is a novel finding.

Reproduction: (Dairy)

P03-003-212

Pre-calving and early lactation factors that predict fertility and milk proteins in the transition dairy cow

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Objectives: This study evaluated the effects of dietary protein degradability (ratio of rumen-undegradable protein (RUP) to rumen-degradable protein (RDP)) and genetic merit (GM) (for milk fat + protein yield) on fertility and production in dairy cows. This study aimed to identify pre-calving and early lactation variables that predict fertility, milk yield, and milk protein yield and composition and explored the hypothesis that low milk protein content is associated with lower fertility.

Materials and Methods: Multiparous Holstein cows (n = 82) of high or low GM were allocated to one of two diets in a 2X2 factorial design. Diets differed in the ratio RUP to RDP (37% vs. 15% RUP) and were fed from 21 d pre-calving to 150 d of lactation. Blood concentrations of beta-hydroxybutyrate, cholesterol, glucose, urea, alpha amino nitrogen (AAN), and NEFA were measured from three weeks before calving. Weekly metabolizable protein (MP) balance was estimated for each cow using CPM dairy (version 3.08; Cornell-Penn-Miner) and metabolizable energy (ME) balance was calculated based on formula described by AFRC (1993). Average blood metabolite concentrations, ME and MP balances, BW and BCS for the 3 weeks before calving, and average milk production variables (milk yield, milk protein and casein yield and percentage) for the first 3 weeks of lactation, were evaluated as indicator variables in statistical models. Casein variant (alpha, beta, gamma, and kappa casein) yields and percentages were determined at weeks 2, 6, and 10 of lactation. The proportion of first services that resulted in pregnancy was explored using logistic regression. Survival analysis was

used to explore the effect of indicator variables on the risk of pregnancy up to 150 d postpartum, with cows separated into quartiles based on their milk protein percentage. Canonical linear discriminant analysis was used to identify traits that differed between groups. Mixed models were used to explore the significance of pre-calving indicator variables on milk yield, and milk protein, casein, and casein variant yield and composition.

Results: A diet high in RUP increased proportion of first services that resulted in pregnancy from 41 to 58%. Increased pre-calving MP balance decreased the proportion of first services that resulted in pregnancy when evaluated in a model containing CN yield, diet, and GM, indicating that the positive effects of a diet high in RUP on fertility may be moderated by retained MP balance in cows with a very positive MP balance before calving. Prepartum MP balance was important for production and reproduction outcomes; while surprisingly, ME balance was not. Cows that produced the lowest quartile of milk protein percentage were 28% less likely to become pregnant during the first 150 d of lactation. Factors associated with lower protein percentage were greater milk production (3.87 L/cow/d more), increased prepartum plasma glucose and calcium, MP balance (497 vs. 414 g/d), and lower blood urea. Increased RUP increased milk yield, and milk protein and CN yields; while, increased GM increased milk yield and tended to increase milk and milk protein yields. Increased GM increased gamma CN yield. Largely consistent associations were found for the effects of indicator variables on CN variant yields and concentrations, with higher BW and AAN resulting in higher yield, but lower concentrations. An increase in cholesterol was associated with decreased CN variant concentrations; while, disease negatively affected CN variant yield.

Conclusions: This study demonstrates the importance of protein metabolism to reproductive performance and supports the hypothesis that low milk protein can be an indicator of poor reproductive performance in the dairy cow.

Reproduction: (Dairy)

P03-003-213

Resumption of ovarian cyclicity and energy balance in Holstein cows in France based on in-line milk measurements

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Objectives: High reproductive performance in dairy herds is a prerequisite for farm profitability. Resumption of post-partum ovarian cyclicity is one of the main limiting factors of reproduction in Holstein cows since fertility is negatively affected by the delay from calving to first ovulation. Most available studies are based on experimental herds, performed in specific conditions. The purposes of this study were to describe the postpartum cyclicity resumption in Holstein cows under current farming conditions in France. We focus on cyclicity resumption, cow energy balance and their effect on reproduction.

Materials and Methods: Data were collected in France during spring 2015 in 21 farms equipped with DeLaval milking robots with a Herd NavigatorND system with DelproND software (DeLaval International, Tumba, Sweden). A total of 1247 cows during the first 90 days of lactation were included. The proportion of primiparous females was

38.3% (478/1247). Progesterone and β -hydroxybutyrate (BHB) were assayed on milk in-line. Progesterone was assayed on average every 2.7 days from 20 to 90 days postpartum (dpp). The delay between calving to the onset of luteal activity (C-LA) was defined as the number of days from calving to the first day with milk progesterone concentration higher than 5 ng/mL. BHB assays, performed every 3 or 4 days during the first 21 dpp, were used to determine the ketosis status. If at least one milk BHB value was greater than 0.15 mmol/L during the first 21 dpp, the cow was declared affected by ketosis. Milk production data were available for each milking. Artificial insemination and pregnancy status were recorded.

Results: Nearly half the cows (45.3%; 565/1247) had resumed cyclicity before 30 dpp and 86.2% (1075/1247) before 60 dpp with significant differences between farms ($p < 0.001$). The proportion (48.9%, 420/859) of young cows (parity 1 or 2) that started cycling before 30 dpp was higher ($p < 0.001$) compared to the proportion of older cows (parity ≥ 3 ; 37.3%, 145/388). Cows that resumed cyclicity before 30 dpp had a lower cumulative milk production over the first 60 dpp than cows resuming cyclicity after 30 dpp (mean \pm sd; 2097 \pm 495 kg vs 2156 \pm 522 kg; $p = 0.05$). In contrast, cumulative milk production over the first 30 dpp had no effect on the resumption of luteal activity ($p = 0.14$). Cows with C-LA lower than 60 dpp (912/1034) were inseminated significantly earlier than those with later C-LA (85.0 \pm 26.5 days vs 105.1 \pm 32.5 days; $p < 0.001$). Similarly, their calving to conception interval was shorter (116 \pm 49.2 days vs 138 \pm 52.6 days; $p < 0.001$).

Overall incidence of ketosis was 19% (237/1247), varying significantly between farms (4.9 to 55.3%, $p < 0.001$). Milk production over the first 60 dpp was significantly higher in cows with ketosis (2087 \pm 495 kg vs 2305 \pm 506 kg). Cows with an early resumption of luteal activity (< 30 dpp) were those with the lowest incidence of ketosis (12.9% vs 24.0% for cows with C-LA > 30 dpp). Cows with ketosis had a longer calving to first insemination interval (86.2 \pm 27.7 days vs 92.7 \pm 28.6 days; $p = 0.005$) and a longer calving to conception interval (116.1 \pm 49.5 days vs 131.8 \pm 51.0 days; $p = 0.006$).

Conclusions: The interval from calving to luteal activity is affected by both ketosis and milk production over the first two months of lactation. In turn, later impact of C-LA on fertility appears of major economic importance. In-line progesterone and BHB assays are very potent tools for dynamic monitoring of reproductive and metabolic status. They especially show the quality of the transition period management: any disturbance evidenced by these indicators needs to be followed by appropriate corrective management solutions. Further analysis of these data will focus on the patterns of postpartum progesterone cyclicity.

Reproduction: (Dairy)

P03-003-214

Reproductive outcomes of a blanket herd synchrony program in New Zealand seasonal dairy farms

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Objectives: Successful blanket herd synchrony programs are useful for dairy farmers seeking to reduce dependence on heat detection or simplify their management system. The objective of this study was to assess the herd level reproductive effects of a whole herd synchrony program with fixed time artificial insemination (FTAI) compared to untreated controls in NZ seasonal dairy farms.

Materials and Methods: Lactating dairy cows from 10 seasonally calving pasture-based dairy herds were enrolled. To be eligible, cows must have calved in the current season and more than 34 days before the farm's planned start of mating (PSM). Cows were excluded if birth ID, breed code, date of birth and latest calving date data were unavailable or if the farmer did not intend to mate them in the current season. Heat detection commenced 35 days prior to the planned start of mating (day 0). The enrolled cows were randomly allocated within farm into control and treatment groups. All cows were body condition scored on enrolment. In the control group, cows that had not been observed in heat by 9 days prior to PSM ("non-cycling" cows) were enrolled in the FTAI program and the remaining "cycling" cows were mated to observed heats after the PSM with no mating intervention. All treatment group cows were enrolled in the FTAI program regardless of whether they had been observed in heat prior to PSM or not. The synchrony program consisted of: Day -9: 1.38g progesterone device (CIDR®) inserted & 100µg gonadorelin acetate injected. Day -2: progesterone device removed, 25mg dinoprost injected & 400IU equine chorionic gonadotrophin (eCG) injected. Day 0: 100µg gonadorelin acetate followed by FTAI 10-14 hours later. All synchronised cows from both groups were submitted for FTAI on the farm's first day of mating. Day of conception was determined by aged pregnancy diagnosis with transrectal ultrasound. The primary outcomes compared were 21, 42 and 70 day pregnancy proportions and time to conception.

Results: 4,180 cows were enrolled. 39% were identified as non-cycling at day -9. As the non-cycling cows in both groups were treated with the synchrony program, the analysis describes the synchrony effect in the cycling cows only. Excluding the non-cyclers, blanket herd synchrony significantly increased the odds of conception within 21 days (OR 1.42 (1.21 - 1.67), P< 0.01) and 42 days after PSM (OR 1.40 (1.17 - 1.69), P< 0.01) and tended to increase the odds of conception within 70 days after PSM (OR 1.12 (0.89 - 1.42) P =0.34). Blanket herd synchrony reduced the mean time to conception of the cyclic cows by 7.5 days (P<0.01).

Conclusions: Blanket herd synchrony allowed the farmers to submit all cows on a single day at the start of artificial breeding, thus simplifying heat detection. It resulted in earlier conception within a seasonal mating program, meaning that the cows will calve earlier the following season and therefore may have a longer lactation. This study showed that, in addition to the farm management advantage, whole herd synchrony also significantly improved the reproductive performance of these herds. The shortened time to conception for the treated cows in this study made the program economically viable in NZ.

Reproduction: (Dairy)

P03-003-216

Selenium Blood Levels Are Related To Placental Retention, Endometritis And Body Condition Score Loss During Postpartum In Holstein-Friesian Cows

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Objectives: To analyze the relationship among Selenium blood levels (Se) since Day 270 of gestation to 4 weeks after parturition and the body condition score (BCS) loss, and reproductive parameters of high producing dairy cows.

Materials and Methods: The study was performed in a commercial farm located in the Northeast of Spain. The farm counts with 850 lactating

dairy cows which are milked 3 times daily and are fed according to the National Research Council recommendations. Forty seven healthy, non-aborting multiparous cows (≥3 lactations) were analyzed. Blood samples were collected from the coccygeal vein at Day 270 of gestation, at parturition and at Days 7, 14, 21 and 28 postpartum (pp). Blood samples were stored at -20°C until analysis. Blood samples were diluted and subjected to an ultrasound bath to break cellular membranes before measure Selenium blood levels through spectrophotometry. The effects of the variables BCS loss (prepartum-Day 14 pp), endometritis and placental retention (PR) on blood Selenium concentrations for all animals were assessed by GLM repeated measures analyses of variance. A binary logistic regression model was built entering PR, endometritis, cyclicity and positive pregnancy diagnosis 28-34 days following first AI as the dependent variable. Independent factors for each dependent variable were: calving season, lactation, inseminating number, bull, days in milk, normalized milk production during the first 305 days in milk of the lactation study period and selenium blood levels at Day 275 of gestation, parturition and Days 7, 14, 21 and 28 pp as independent. All statistics procedures were performed using the SPSS package version 18.0 (SPSS Inc., Chicago, IL, USA) with significance set at P<0.05.

Results: Mean (±SD) blood Selenium concentrations were 128.8±26.3, 140.1±31.2, 128.7±31.3, 116.7±26.6, 111.2±23.7, 109.1±28.1 for Day 275 of gestation, parturition and Days 7, 14, 21 and 28 pp, respectively. Cows losing more than 0.75 points of BCS (n=23) showed higher Se during the whole study period than the remaining animals (n=24) (between subject effect P<0.0001). Endometritis group (n=13) showed higher levels of Se during the study period (between subject effect P<0.0001). Eighteen cows suffered PR. Based on the odds ratios, the likelihood of PR increased in cows with high Se at parturition (by a factor of 1.02) compared to the remaining cows.

Conclusions: Holstein-Friesian multiparous cows showing high levels of selenium in blood during prepartum, parturition and postpartum are losing more BCS, suffer more endometritis during postpartum and more placental retention than the remaining animals.

Reproduction: (Dairy)

P03-003-217

Analysis of Systemic Changes in Cows with Subclinical Endometritis

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Objectives: All cows experience an ascending influx of bacteria through the cervix during and soon after calving. While the majority clear the microbes, a significant proportion (~30%) fails to do so, leading to prolonged endometrial inflammation, uterine disease and subfertility. One of the major causes of decreased fertility postpartum is endometritis and the diagnosis of sub-clinical disease is limited by the lack of sensitive and specific biomarkers. We hypothesise that early systemic immune changes associated with sub-clinical endometritis may provide prognostic indicators of cows which are likely to develop uterine disease.

Materials and Methods: Uterine cytology, mucus scores and peripheral blood evaluations from postpartum cows (n=139) were performed at 7 and 21 days postpartum (DPP). Cytological classification was based on

an 18% polymorphonuclear (PMN) cell cut off at 21 DPP to diagnose subclinical endometritis. Vaginal mucus was evaluated on a scale of 0-3; a score of 0 or 1 indicated the absence of clinical endometritis. Two distinct groups were identified; resolvers (n=10) and non-resolvers (n=10). Both had a vaginal mucus score of either 0 or 1 at both time points and high PMN infiltration 7 DPP (45.5% and 54.1%, resolvers and non-resolvers respectively). However, 21 DPP the resolvers had an average of 8.9% PMN compared to 57.3% PMN for non-resolvers.

Results: There were no significant differences in plasma glucose, non-esterified fatty acid, betahydroxybutyrate and urea levels between resolvers and non-resolvers at any time point. Haematological analysis revealed significant differences in relative neutrophil (P<0.001) and lymphocyte (P<0.001) numbers systemically at 7 DPP prior to the observed uterine changes at 21 DPP. Pre-calving the non-resolvers had increased relative gene expression of immune receptors, cell surface markers - (TLR2 [P=0.012], CD68 [P=0.047], CD80 [P=0.025] and CD172 [P=0.009]); as well as pro-inflammatory cytokines - (TNF [P=0.06] and IL6 [P=0.055]) compared to cows which had resolved inflammation by 21 DPP. At 7 DPP, CD172 and TNF were also significantly increased in non-resolving cows. Interestingly resolvers only had significantly (P<0.001) increased relative gene expression of immune receptor TLR4, 7 DPP.

Conclusions: Results show evidence of systemic immunological changes in cows at 7 DPP, significantly in advance of the usual diagnosis of subclinical endometritis. Furthermore, significant changes in gene expression as early as 62 days pre-calving in cows that develop disease suggests that immune dysregulation may begin before calving. The identification of systemic biomarkers associated with subclinical endometritis will improve reproductive health in cattle by aiding in early detection and prevention of uterine disease.

Reproduction: (Dairy)

P03-003-218

Redefining the inflammatory gene signature associated with endometritis in bovine endometrial epithelial cells

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Objectives: Bacterial contamination of the uterus is ubiquitous in cattle after calving, and the resulting inflammation can lead to uterine disease if not resolved. Recently our group uncovered a prolonged inflammatory gene signature in endometrial biopsies, associated with the development of endometritis. Endometritic cows displayed consistent inflammation at 7 and 21 days postpartum (DPP), whilst healthy cows showed a decline in inflammatory mediators by 21 DPP. This study aims to validate these results in a larger set of animals and compare the inflammatory signature of the mixed uterine cell population in biopsies with endometrial epithelial cells.

Materials and Methods: Previous work involved collection of biopsy samples from n=9 healthy and n=6 subclinical cows at 7 and 21 days postpartum (DPP). RNA was extracted from the biopsy samples and RNA quality was assessed using an Agilent Bioanalyzer. Following

cDNA synthesis, gene expression analysis was carried out by RT-qPCR. To further investigate gene expression in a single cell population, duplicate cytobrushes were used to collect endometrial epithelial cells from the uterus at 7 and 21 DPP, from a total of 139 cows. Cytological assessment classified animals into groups of high or low inflammation at each time point, based on an 18 % polymorphonuclear (PMN) cell cut-off. Total RNA will be extracted from n=47 cows (n=26 subclinical and n=21 healthy) and relative gene expression analysis performed using RT-qPCR.

Results: Gene expression analysis in uterine biopsy samples supports the presence of a prolonged inflammatory profile associated with endometritis. Greater expression of pro-inflammatory cytokines such as IL1 α , IL1 β and IL18 was observed in subclinical cows compared to healthy cows. Additionally, up-regulation of β -defensin antimicrobial peptides and S100 genes at 21 DPP suggests that infection is on-going in these cows and has not been resolved. Significant differences in histone deacetylase enzymes at 7 and 21 DPP suggest that epigenetic mechanisms may also have a role to play in the postpartum immune response in cattle. Profiling of endometrial epithelial cells will investigate the expression of similar cytokines and antimicrobial peptides and provide additional information on the mechanisms involved in this immune response.

Conclusions: Preliminary findings support the theory of a prolonged inflammatory signature in cows with subclinical endometritis. Investigating this profile in endometrial epithelial samples will allow us to observe the contribution of the epithelial cell to this response, improving our understanding of the innate immune system in postpartum cattle and aiding the development of future intervention strategies for endometritis.

Reproduction: (Dairy)

P03-003-219

Is crossbreeding a viable option for improving fertility in Australian dairy herds?

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Objectives: Cow fertility has steadily declined in many countries including Australia in the last thirty years, impacting on both profitability and sustainability. A long-term solution to poor fertility is to select for increased milk production whilst including genetic merit for fertility in breeding plans. However the restoration of fertility to previous levels through the genetic route could take many years and thus alternative approaches need to be promoted and identified through research, development and extension. The use of crossbreeding as a short-term solution for poor fertility in Australian herds has been assessed.

Materials and Methods: Milk production is strongly seasonal in major SE Australian pasture-based regions with peak production being achieved at times of peak growth. The seasonality of the industry means many herds need to calve within a period of four months or less with rebreeding at 12-month intervals to achieve a concentrated calving pattern for the following season. Reproductive performance is therefore essential to maintaining farm sustainability and profitability by concentrating the calving pattern to maximise milk yield and feed utilisation.

In 1996 the InCalf program was created to monitor the reproductive performance of Australian dairy cows. A range of reproductive parameters including 6-week in-calf rate, submission rate and conception

rate achieved in 124 seasonally calving herds were established by the study. A second InCalf project, undertaken in 2009, analysed 74 herds over a ten year period from 2000 to 2009 to determine whether fertility had declined over that period. The study compared figures from the 74 herds to the base parameters derived in the original InCalf project.

Only a small number of Australian studies have looked at the implications, benefits and profitability of crossbreeding under local, pasture-based conditions. The studies were designed to assess whether the reproductive performance of crossbreds, including New Zealand cross Holstein-Friesian cows, in predominantly Holstein-Friesian herds, was superior to that achieved by Holstein-Friesians in terms of submission rate, conception rate, in-calf rate and numbers not pregnant at the end of the breeding period.

Results: The crossbreeding studies showed that crossbred Australian cows had better fertility and longevity than Holstein-Friesian cows with the crossbred herds more profitable than pure-bred Holstein-Friesian herds. This was due to their superior reproductive performance, more compact calving pattern, longer survival in the herd and higher stocking rate. The crossbreds had an 8% higher first service conception rate and six-week in-calf rate and a 5% lower not in-calf rate. Moreover, although Holstein-Friesian cows produced more milk, New Zealand cross Holstein-Friesian cows were shown to have an 8% superior 14-week in-calf rate and a 9% lower not in-calf rate.

The Australian InCalf analysis found all breeds including crossbreds experienced a significant decline in fertility over the period from 2000 to 2009. The typical 6-week in-calf rate decreased by 1% per year with submission and conception rates down by 0.6% and 0.7% per year respectively. However the Jersey Holstein-Friesian first crosses and back crosses had superior fertility to the Holstein-Friesian and Jersey cows in the study herds.

Due to the limited number of crossbreeding studies that have been undertaken in Australia and the increasing interest in crossbreeding programs, Dairy Australia funded a study that commenced in 2015 to assess the use of crossbreeding in Australian herds. The research will determine what breeds are being used, their relative performance and how breeding plans are managed including comparing 2-way with 3-way crosses to provide managers with information on why and how to manage a crossbred herd.

Conclusions: Although selection within breed could address various limitations in dairy genetics over time, combining it with crossbreeding to maintain genetic diversity will improve profitability, longevity and animal welfare. Managers of seasonal calving herds should consider an aggressive approach to the selection of bulls with positive daughter fertility whilst implementing a crossbreeding plan that utilises high genetic merit bulls using two or three different breeds. Establishment of such a crossbreeding program is a serious, long-term commitment requiring selection of specific breeds as well as a type of crossbreeding system to suit the individual.

Reproduction: (Dairy)

P03-003-220

Influence of Oxytocin Treatment on the Length of Estrus Cycle in lactating Dairy Cows

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Objectives: There is some evidence in elder literature, that application of oxytocin during the early luteal phase influences cycle length in cattle. Armstrong and Hansel (1959) and Donaldson and Takken (1968) reported that treatment with oxytocin during the first week of the estrus cycle led to premature luteolysis between 8 - 12 days after ovulation. The objective of the present study was to verify, if repeated applications of 20 IU oxytocin shorten the estrus cycle.

Materials and Methods: Ten multiparous, lactating Holstein Friesian cows were examined every two days and from day 10 after ovulation on daily via transrectal sonography (8 MHz transrectal probe, LCS scanner 100, Pie Medical®, Maastricht, NL) for follicular growth, time of ovulation and subsequent development of the corpus luteum. From day 10 of the estrus cycle on blood samples were collected to determine serum concentrations of progesterone and estradiol-17β. In group 1 (control; n = 10) for each cow a non-influenced estrus cycle was observed. During the following cycle (group 2; n = 10) each cow received 20 international units (IU) oxytocin intravenously from day 2 until day 6 after ovulation. The parameters cycle length, maximum diameter of the dominant follicle, concentrations of estradiol-17β and progesterone and expression of estrus behaviour were evaluated by means of single-factorial variance analysis.

Results: The mean length of the estrus cycles was 21.9 ± 1.6 days in group 1 and in 21.1 ± 2.1 days in group 2 and thus was not influenced by oxytocin treatment (p = 0.19). The diameter of the preovulatory follicle was not influenced either by the application of oxytocin (15.9 ± 1.6 mm in the untreated group 1 and 16 ± 2.1 mm in group 2, p = 0.98). Also the periovulatory concentrations of progesterone and estradiol-17β and the expression of estrus signs showed no significant difference between both groups (p > 0.05).

Conclusions: No influence of oxytocin treatment on all documented parameters could be observed. The commonly used dosage of 20 IU Oxytocin for the treatment of udder diseases or difficult milking does not influence observed parameters of the estrus cycle in dairy cows.

Reproduction: (Dairy)

P03-003-221

Study on a corpus luteum with a cavity in dairy cows

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Objectives: The role of a corpus luteum with a cavity (CLcav) in cows has still been discussed (Kastelic 1990, Kot, 1999). The question on its potential influence on fertility in cows is far open (Perez-Marin, 2009, Hatvani, 2013). The aim of our study was to show the relations between some factors and frequency of the corpus luteum with the cavity (CLcav).

Materials and Methods: Four hundred fifty nine corpora lutea at different stages of the cycle during pregnancy and in non-pregnant cows were ultrasonographically evaluated. A portable ultrasound instrument (Draminski iScan) with a linear probe (7.5 MHz) was used. Ultrasonograms of the corpus luteum (CL) were collected during the routine ultrasound examination and with respect for the veterinary ethics. All CL were measured (diameter, size, volume). The corpus luteum with the cavity was defined as an echodense structure with a non-echodense cavity ≤ 20 mm in diameter (Hatvani, 2013).

Results: Generally, 239 cows and 190 heifers were examined. Ultrasonograms of 459 corpora lutea (CL) were analysed. The mean diameters, sizes and volumes of CL were: 23.23 mm (11 – 43 mm), 465.81 mm² (89– 1412 mm²) and 7999.83 mm³ (694 – 47565 mm³). On average, the CL on the right ovary were smaller in size and volume than CL on the left ovary, however the average diameter of CL on the right ovary was bigger than on the left ovary (23,19 v. 23,18 mm, 458.66 v. 472,24 mm² and 7844,71 v. 8149,16 mm³). The cavity in the corpus luteum was stated in 27.1% more often on the right than on the left ovary (27,1 v. 27%). The average diameter of CLcav was bigger than CL compact (24,45 v. 22,77 mm). The average CLcav had also more luteal tissue than CL compact (7979,59 v. 7634,65 mm³). The corpus luteum with the cavity was more frequent in heifers than cows (35,8 and 20,7%). The average cavity was the biggest in days 6-8 after oestrus, smaller in days 9-14 and the smallest in days 15-19 after oestrus (6-8d:10,09 mm v 9-14d:9,32 mm v.15-19d: 8,5 mm). The percentage of cavities in cows examined for pregnancy was 15,4 for pregnant, whereas for non-pregnant 25,6%. In the presence of two or more CL on one ovary, cavities occurred in 20,5% of double CL and in none of triple CL. The CL with the cavity seems to be the most frequent in autumn, the least likely in winter (spring: 24,7%, summer 26,1%, autumn: 36%, winter: 4,2%). In three CL there were two separated cavities in one CL (0,7%).

Conclusions: Further investigations are necessary to explain differences in frequency of CLcav in respect to the season. Seasonal differences in their frequency are vague. They may have some connection with heat stress and hypoxia.

Reproduction: (Dairy)

P03-003-222

Effect of postpartum eCG administration in dairy cows calved during the hot season on subsequent fertility after first timed artificial insemination

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Objectives: Heat stress affects especially fertility of high producing dairy cows. Cows calving during the hot season show a significant delay in the onset of reproductive activity, determining a seasonality of the production, which limits the optimization of profitability. It has been reported that early application of eCG postpartum animals could accelerate the onset of ovarian activity and uterine involution.

The objective of this work was to evaluate the effect of the application of eCG in the early postpartum (at days 11-17 postpartum) on the fertility after first timed AI of heat stressed high producing dairy cows.

Materials and Methods: a total of 400 freshen cows from one single commercial farm (1217 lactating cows) with calvings between 27th June and 3rd of October received randomly 500ui of eCG (eCG-Group; n=213) or saline solution (Control-Group; n=187) at day 11-17 postcalving. At day 96.34±9.88pp a synchronization protocol based on the Double Ovsynch-protocol was applied and all cows were timed artificially inseminated. Ultrasonography examination was performed at day 0 (eCG administration), every 7 days during 3 weeks, and during synchronization and 7 days after AI, in order to assess ovarian activity assessed by presence of a corpus luteum (CL) and or dominant follicle (DF). At day 30 postpartum subclinical endometritis was assessed through cytobrush

uterine cytology (cut-off for Polimorphonuclear Neutrophils MN ≥20%). Pregnancy diagnosis was performed at day 30 and 60 after AI.

Results: Average lactations/cow (2.33 ±1.34), DIM at first AI (96.33±9.88), average milk yield at AI (41.38±7.74L), inseminator distribution (2 inseminators) and used bulls (6 different bulls were used) did not differ between treatments (P>0.05).

The ovarian activity increased with time (with 4.2, 30.2 and 48.4% of animals with CL at weeks 1, 2 and 3 post-eCG, respectively, with a total of 55.1% showing a CL at the beginning of the synchronization protocol. At the time of prostaglandin administration just 31.4% showed a CL. No differences were observed between experimental groups.

At day 30 postpartum 7.7% showed subclinical endometritis (without statistical differences), although the percentage of animals with a hypercogenic intraluminal content (probably purulent content) showed a tendency to be lower in the eCG-Group (21.4 vs. 16.8%; P=0.15). The prevalence of clinical endometritis showed a tendency to be lower in the eCG-Group at 2 weeks after eCG administration (26.2 vs. 19.2%; P=0,059).

Fertility at first AI was 34.9 and 30.4% at 30 y 60d, respectively with no significant difference between experimental groups (day 30 postAI 34.9 vs. 32% and Day60 postAI 30.4 vs. y 28.3%, for eCG-Group and Control. respectively; P>0.2).

Conclusions: Early postpartum application of eCG in heat stressed dairy cows could have benefited slightly endometrial status of the animals, but this did not induce any beneficial effect on the ability to become pregnant at day 30, nor at day 60 after first timed AI. Hence, other strategies should be used in order to mitigate the effect of heat stress during postpartum on fertility of high producing dairy cows.

Reproduction: (Dairy)

P03-003-223

Predicting the onset of stage two of calving in Holstein-Friesian heifers

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Objectives: Especially heifers suffer from a high prevalence of dystocia. Dystocia is associated with major pain in the dam and an increased risk of stillbirth in calves. One requirement to prevent and manage dystocia in heifers is to recognize the onset of stage 2 of calving. Therefore the objective of our study was to predict the onset of stage 2. We set out to test the predictive value of six signs of imminent parturition (SIP) and the alteration of the broad pelvic ligaments and filling of the teats.

Materials and Methods: Prior to the experiments a systematic literature search was conducted in which six signs of imminent parturition were identified to be plausible parameters to indicate the onset of stage 2 (i.e., tail raising, scurrying, turning the head towards the abdomen, clear vaginal discharge, bloody vaginal discharge and lying lateral with abdominal contractions). The experiments took place on a commercial dairy farm in Germany milking 2,250 cows. In the first experiment the inter-observer reliability of SIP was tested. A total of 22 Holstein-Friesian heifers around calving were included. Two investigators observed each heifer for 15 seconds once per hour walking behind the cows in the pen. In the second experiment SIP were observed every hour 24 hours from 267 d before calving to parturition. 37 Holstein-Friesian

heifers were included. Additionally to SIP, relaxation of the broad pelvic ligaments (PL) and filling of the rear teats (TF) were examined twice a day and evaluated on a 4-point scale. We calculated a receiver operating characteristic (ROC) analysis for a combination of SIP. One score point was given for the occurrence of each SIP meaning it ranged from 0 to 4. Besides a receiver operating characteristic analysis was conducted for the combination of score points for PL and TF.

Results: Cohen's kappa results for the inter-observer reliability were moderate until almost perfect, ranging between 0.507 and 0.912. The cut-off value for occurrence of SIP was >1 with 83.8 sensitivity and 99.2 specificity. A proportion of 99.2 % of heifers having a score under the cut-off value was correctly diagnosed as not calving. Prediction of calving was possible in 36.5 % of calving events. The results of the area under the curve (AUC) for the combination of PL double weighted and TF was about 0.8, implying that in 80% of the examinations a calving heifer will have a higher PL and TF score than a non-calving one. As the results of SIP and PL TF of the negative predictive value were considerably higher than the positive predictive value, we contemplated predicting no calving. The accuracy of excluding calving for the next 12 hours was 96.8% for 29 heifers examined from day 269 days carried calf until parturition corresponding with a cut off value >5 and a 68.97 sensitivity and 87.91 specificity. A binary logistic regression analysis was conducted to determine the effect of group (i.e., late pregnancy vs. 24 h before onset of stage 2) on the occurrence of SIP. Tail raising, clear vaginal discharge and bloody vaginal discharge were more likely to occur 24h before calving than in late pregnancy ($P \leq 0.001$). While there was a difference in detecting imminent parturition between hourly observation and observation every 3h, 4h, 5h, and 6h, respectively ($P \leq 0.05$), there was no difference between hourly observations and observation every two hours ($P = 0.139$).

Conclusions: Prediction of calving using SIP or the evaluation of PL and TF is challenging. Nevertheless, it is useful to exclude the possibility of calving. Knowing which cow will not calve during next 12 h facilitates monitoring of cows around parturition. The appearance of SIP was very individual regarding the different SIP and the time of occurrence. We could not find a difference between hourly observation and observation every second hour. Hence, we suggest to observe pregnant heifers every 2 hours for tail raising, clear and bloody vaginal discharge and lying lateral with abdominal contractions.

Reproduction: (Dairy)

P03-003-224

The role of disease in dairy herd reproductive performance

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Objectives: This abstract summarises the approach and findings of a number of studies which collectively aimed to explore associations between clinical disease events and reproductive performance, both at the level of the individual cow and the herd. The overall objective was to evaluate the potential improvement in a herd's reproductive performance which might be expected as a result of implementing measures to reduce the levels of the major endemic diseases (e.g. mastitis and lameness).

Materials and Methods: Routinely collected herd management data (including cow identity data, insemination and pregnancy diagnosis events and clinical disease records) was collected from a total of 468

dairy herds in England and Wales. Multilevel discrete time survival models were used to explore associations between disease events and reproductive outcomes at lactation level. This statistical approach allowed control for potential measured (e.g. milk yield) and unmeasured (e.g. differences in heat detection standard between herd) confounding variables. A stochastic simulation model was then developed and used to explore the potential for a herd's level of disease to influence its overall reproductive performance. Additionally, the same dataset was used to explore associations between lactation-level reproductive performance and periparturient disease events (e.g. uterine bacterial disease, hypocalcaemia and cystic ovarian disease), using multilevel logistic regression models. A similar dataset was used to explore associations between antibody status for Johne's disease and lactation level reproductive outcome, again using multilevel logistic regression.

Results: Discrete time survival models revealed some statistically significant and relatively large associations between mastitis/lameness events and reproductive outcomes. For example, the odds of a cow becoming pregnant in a given period of time were reduced by 42% in the seven days following a clinical mastitis event. Similarly, odds of pregnancy per unit time were reduced by 24% in the fortnight before or after a clinical lameness case. However, simulation models revealed that, despite these effects, there would be very few situations in which a herd's overall reproductive performance would be altered by a clinically meaningful amount in response to changes in incidence rate of either mastitis or lameness (or in subclinical mastitis as measured by individual cow somatic cell count).

Preliminary results suggest that the odds of a cow becoming pregnant before 100 days in milk (DIM) were reduced where the lactation included a record of a case of retained foetal membranes, uterine bacterial infection or cystic ovarian disease (with reductions in odds of pregnancy by 100 DIM of 31%, 48% and 67% respectively). Odds of pregnancy before 100 DIM were reduced by 79% in cows with a positive Johne's antibody status compared to negative cows. A much smaller association between Johne's status and the odds of insemination at less than 80 DIM suggested that the association with overall reproductive outcome was not explained simply by farmers electing not to inseminate positive cows. Further work is required to interpret these results in the same context as those for mastitis and lameness.

Conclusions: Whilst there are statistically significant associations between reproductive outcomes and clinical records of both lameness and mastitis, improving control of these diseases at herd level is highly unlikely to make a measurable or clinically significant difference to the herd's level of reproductive performance. Preliminary work on the associations between reproductive outcomes and both periparturient disease and Johne's disease status suggest that both have the potential to impact fertility at lactation level to a large degree. Further work would be required to evaluate the potential significance of this at herd level.

Comments: Note: elements of the work summarised in this abstract has previously been published; novel provisional results from further work are also included.

Reproduction: (Dairy)

P03-003-225

Accuracy and intensity of estrus detection with activity monitoring systems for lactating dairy cows

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Objectives: Activity monitors are becoming a widely used tool for estrus detection in dairy cows. The objective of this study is to assess the intensity and accuracy of estrus detection with automated activity monitoring systems.

Materials and Methods: Three commercial dairy farms with an activity monitoring system (Heatime, SCR Inc, or Afi PedoPlus, Afikim) that was used for essentially all inseminations between 50 and 80 days in milk (DIM) were enrolled in this observational study. Herds were visited once weekly. Blood samples were collected at weeks 5, 7, and 9 postpartum and from a subset of cows on the day of insemination, to measure serum progesterone concentration. Cows were examined at week 5 for purulent vaginal discharge (PVD). Lameness and body condition were scored at week 7. Cows were classified as anovular if all 3 blood samples from weeks 5, 7, and 9 had progesterone < 1 ng/ml.

Results: Overall, 6.7% of cows were anovular (range of 3.6% to 8.2% among farms). Of these cows, 39% had a lameness score of 3 or above (1 to 5 scale) and 29% had PVD. Among cows that reached 80 DIM (n=419), 14% had not been inseminated by 80 DIM; of these, 12% were anovular, 23% were lame, 21% had PVD and 4% had a BCS of <2.5%. Among 352 blood samples taken on the day of insemination (all services; 212 signaled by the activity system and 140 by Ovsynch), 8% and 3%, respectively, had progesterone > 1 ng/ml i.e., the cows were not in estrus.

Conclusions: In this sample, the accuracy of detection of estrus by automatic activity monitoring systems under commercial conditions was high. However, a subset of cows would likely require intervention for timely first insemination and only a fraction of these were truly not cyclic.

Reproduction: (Dairy)

P03-003-226

Effect of two different bovine somatotropins on reproductive outcomes of dairy cows in Brazil

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Objectives: Bovine somatotropin (bST) is recognized as a stimulus for increased milk production. The objective of this trial was to assess the reproductive outcomes of a high-producing dairy cattle herd in response to two commercial sources of bovine somatotropin (bST) administered every 14 days totalizing 16 applications. Our hypothesis was that both bST have the same effect on reproductive outcomes.

Materials and Methods: Eighty Holstein cows from a commercial farm located in the south region of Brazil, averaging 1.76 ± 1.16 lactations, 77.1 ± 21.9 days in milk (DIM) and 37.5 ± 6.5 kg/d of milk were randomly assigned to one of 2 treatment groups using milk production during the 7-day pretreatment period as the blocking criterion. Treatments consisted of 16 consecutive subcutaneous injections of 500 mg of bST (Boostin, MSD Saúde Animal, Brazil) or 500 mg of bST (Lactotropin, Elanco Saúde Animal, Brazil), both administered at a 14-day interval. All cows were milked 3 times a day and received the same total mixed ration fed 5 times daily, consisting of corn silage, ryegrass silage, corn grain ground, soybean meal, whole cottonseed, soybean hulls, urea, minerals, and vitamins. Cows were housed in free stalls. Before the beginning of the trial, 7.5% (6/80) of cows were pregnant (2.5% vs 12.5%, respectively for Boostin and Lactotropin). Data were analyzed with the SAS procedure

mixed with a model containing the continuous effect of the covariate and the fixed effects of block, treatment, day, number of administrations and the interaction between treatment and day and treatment and number of applications. Data were analyzed using the GLIMMIX procedure of SAS software version 9.3.

Results: The data are presented as percentage or mean ± standard error after correction using the final models. Pregnancy rate (%) at the end of the trial was not significantly different (50.0% vs. 55.0%; P=0.32) between both somatotropins. Also, the number of services to pregnancy (3.45 ± 0.54 vs. 3.49 ± 0.40; P=0.93), the interval between calving to pregnancy (129.7 ± 22.9 vs. 130.4 ± 17.1; P=0.97) and the projected intercalving interval (13.5 vs 13.5 mo, P=0.97) were not different between Boostin and Lactotropin, respectively.

Conclusions: These data indicate that there is no statistical significant difference in the effect of the two different somatotropins on reproductive performance (pregnancy rate, number of services to pregnancy, interval calving to pregnancy, and projected intercalving interval) of high-producing dairy cows.

Reproduction: (Dairy)

P03-003-228

Impact of heat stress on AI and natural service breeding programs in the temperate climate

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Objectives: Reduced reproductive performance of dairy cows in combination with reduced libido and semen quality of bulls during periods of heat stress can cause important economic losses to the dairy industry. Therefore, the breeding type used in periods of heat stress should be considered to maximize reproductive performance.

The objective of the study was to determine the influence of short and long term exposure to heat stress on the CR of lactating dairy cows in different natural service and artificial insemination (AI) breeding programs. Furthermore the relationship between breeding type and parity was determined.

Materials and Methods: The retrospective study was conducted on a commercial dairy farm in Sachsen-Anhalt, Germany from May 2010 to October 2012. The herd consisted of 1,150 Holstein dairy cows with an average milk production of 10,345 kg. The barn was positioned in a NE-SW orientation with open ventilation and a mechanical fan-system. The voluntary waiting period was set at 55 d postpartum. Between 35 and 49 d cows received an initial injection of 25 mg PGF_{2α} (Dinoprost, Dinolytic, Zoetis Deutschland GmbH, Berlin, Germany) and a second injection of 25 mg PGF_{2α} 2 weeks later to regress the corpora lutea. Cows that showed estrus after the second injection of PGF_{2α} were artificially inseminated or received natural service by a bull. Pedometers (Milklime, Gariga di Podenzano, Italy) and visual observation were used to detect estrus. Inseminations were performed within 12 h after detection of estrus with frozen-thawed or fresh semen. Pregnancy diagnoses were performed 35 to 42 d after the day of breeding with transrectal ultrasonography by the herd veterinarian.

Ambient temperature and relative humidity within the barn were recorded using a Tinytag Plus II logger (Gemini loggers Ltd, Chichester, UK) and used to calculate the temperature-humidity-index (THI) according to the equation reported by the NRC (1971):

$THI = (1.8 \times AT + 32) - ((0.55 - 0.0055 \times RH) \times (1.8 \times AT - 26))$.

Short term heat stress was defined as a mean THI ≥ 73 at the day of breeding and long term heat stress was defined as a mean THI ≥ 73 in the period from d 21 to d 1 before day of breeding.

Results: The dataset contained 5,192 breeding records from 1,537 lactating dairy cows on a single dairy farm. The overall conception rate (CR) obtained was 33.0% and average open days were 129.3 d. Inseminations with frozen-thawed and fresh semen were conducted in 80.6% and 5.6% of the cows, respectively and 13.8% of the cows were bred by natural service. Minimum, maximum, and mean temperature-humidity index during the study period were 34.6, 83.7, and $60.4 \pm 8.5\%$, respectively.

Multiparous cows bred by AI with frozen-thawed semen were 22% less likely to get pregnant than primiparous cows. Cows bred by AI with frozen-thawed semen exposed to long term heat stress were 63% less likely to get pregnant than cows not exposed to heat stress. Cows bred by AI with frozen-thawed semen receiving ≥ 4 services were 15% less likely to get pregnant than cows receiving 1 service. There were no significant interactions between heat stress and the variables parity and number of services.

Multiparous cows bred by AI with fresh semen were 67% less likely to get pregnant than primiparous cows. Cows bred by AI with fresh semen exposed to short term heat stress were 80% less likely to get pregnant than cows not exposed to heat stress. There were no significant interactions between heat stress and parity.

In the multivariable logistic regression model investigating cows bred by natural service, the variables heat stress ($P = 0.21$), parity ($P = 0.98$) and number of services ($P = 0.24$) had no significant association to CR.

Conclusions: The present study indicates, that the likelihood to get pregnant is reduced by short and long term heat stress depending on the breeding type. Especially CR of cows inseminated with fresh semen are negatively affected by short term heat stress and cows inseminated with frozen-thawed semen are negatively affected by long term heat stress. The CR of cows bred by natural service were not affected by short and long term heat stress. Therefore climate conditions should be considered in the selection of breeding strategies to optimize AI and resulting CR. During periods of short term heat stress the application of fresh semen needs to be improved.

Reproduction: (Dairy)

P03-003-229

Effect of insemination after estrous detection on pregnancy per AI and pregnancy loss in a Presynch-Ovsynch protocol: A meta-analysis

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Objectives: Presynchronization of cows with 2 injections of prostaglandin administered 14 d apart (Presynch-Ovsynch) is a widely adopted procedure to increase pregnancy per artificial insemination (P/AI) for the first service. Two different strategies can be observed. Either all cows are time-inseminated (onlyTAI) or cows detected in estrus after the second PGF_{2a} injection are inseminated and the remaining cows without signs of estrus will be subject to TAI (EDAI+TAI). The objective of our study was to evaluate the effect of insemination after estrous

detection during a Presynch-Ovsynch protocol for first service on fertility in lactating dairy cows.

Materials and Methods: A systematic review of the literature was performed. Two statistical approaches were conducted using a fixed or a random effects meta-analysis based on the heterogeneity among the experimental groups. Reproductive outcomes of interest were P/AI measured on d 32 (28 to 42) and pregnancy loss between d 32 and 60 (42 to 74) of gestation. In approach 1, 3 randomized controlled studies including 1,689 cows with the primary objective to evaluate the effect of insemination after estrous detection in a Presynch-Ovsynch protocol were used. In approach 2, cows from approach 1 and cohorts from another 17 experimental groups including 8,124 cows submitted to first AI using a Presynch-Ovsynch protocol were used. Information regarding pregnancy loss was available for 5,200 cows.

Results: In approach 1, the incorporation of insemination after estrous detection decreased the odds of pregnancy by 35 % (OR = 0.65; 95% CI = 0.53 – 0.80) on d 32 after AI using a fixed effects model. There was no effect on pregnancy loss on d 60 of gestation (OR = 0.88; 95% CI = 0.55 – 1.43). There was no heterogeneity among the 3 studies regarding P/AI and pregnancy loss.

In approach 2, the overall proportion of P/AI was 30.9% (95% CI = 26.71 – 35.28; n = 2,400) and 41.7% (95% CI = 39.76 – 42.01; n = 7,413) on d 32 after AI for EDAI+TAI and onlyTAI, respectively. The overall proportion of pregnancy loss was 11.7% (95% CI = 6.11 – 18.8; n = 1,811) and 9.6% (95% CI = 6.37 – 13.33; n = 3,389) on d 60 after AI for EDAI+TAI and onlyTAI, respectively. There was substantial heterogeneity among the experimental groups regarding P/AI and pregnancy loss.

Conclusions: Based on a meta-analysis considering 20 manuscripts including 27 herds and 9,813 AI we compared 2 different management strategies for first service using a Presynch-Ovsynch protocol. Cows with 100% TAI after completing a Presynch-Ovsynch protocol had more P/AI compared to cows that were inseminated after estrous detection or received TAI for cows showing no signs of estrus. The results of this meta-analysis are based on a large number of herds with different managerial conditions and seem to be applicable to high producing dairy herds under confinement housing.

Reproduction: (Dairy)

P03-003-230

Distribution of inflammation within the endometrium of dairy cows

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Objectives: Aims of the present study were twofold: 1) to compare the distribution of polymorphonuclear cells (PMNs) in 8 pre-defined locations of the endometrium, and 2) to compare the PMN counts between the superficial endometrium (epithelium adjacencies) and PMNs present in the stratum compactum.

Materials and Methods: Holstein-Friesian cows (n= 32) from a single dairy farm at 315±173 days in milk were selected for the present experiment. The day before cows were slaughtered, a complete reproductive examination was performed to discard any type of clinical disease. After slaughtering, reproductive tracts were collected, and the

endometrium was sampled at eight predefined locations. Locations were as follows: (1) tip of the left horn, (2) median part of the left horn, (3) basis of the left horn, (4) left side of the uterine body, (5) right side of the uterine body, (6) basis of the right horn, (7) median part of the right horn, and (8) tip of the right horn. At each location, endometrial biopsies and cytology samples were taken with an 8.0 mm biopsy punch and a cytobrush, respectively. Histopathology samples were stained with Naphtol-AS-D-chloracetate-esterase, while cytology samples were stained with Diff-Quick®. Histopathology slides were evaluated by counting and averaging PMNs in 5 high power fields while cytology samples were evaluated by counting and averaging PMNs in 10 high power fields. For the histopathology samples, PMN counts were differentiated as: PMNs in direct contact with the epithelial cells of the endometrium (PMN-EP), and PMNs present deeper in the stratum compactum (PMN-SC). Summation of both countings was referred to as PMN total (PMN-T). A Poisson mixed effect model was used to analyze the distribution of PMNs within the uterus, and the Tukey method to assess the difference between the estimates of the 8 pre-defined locations (PMN-T), and between PMN-EP and PMN-SC.

Results: In total, 512 acceptable sample slides were obtained: 256 biopsies and 256 cytologies; representing the 8 predefined locations of the 32 uteri. Significantly ($P < 0.05$) higher PMN counts were found in the deep stratum compactum (PMN-SC), in comparison to the superficial endometrium (PMN-EP), and the cytology samples. However, no differences were found in the number of PMNs between cytology and PMN-EP ($P > 0.05$). Significant differences ($P < 0.05$) concerning the number of PMNs were found between the different locations, with higher PMN counts in locations 4 and 6.

Conclusions: Closing, the adjacencies of the corpus uteri should be considered as the preferred location to take uterine biopsies or cytobrush samples in cows, since more PMNs were found in these regions. An uneven distribution of PMNs was found between PMN-EP/cytology and PMN-SC, suggesting that when cytology samples are taken, only the superficial layer of the endometrium is sampled and evaluated.

Reproduction: (Dairy)

P03-003-231

The use of artificial insemination in dairy herds in the Northwestern Rio-grandense mesoregion, Brazil

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Objectives: The objective of this study was to determine the association between the use of artificial insemination and other factors on productive performance of dairy cows in the Northwestern Mesoregion of Southern Brazil.

Materials and Methods: Data were obtained through questionnaires with milk producers of the Northwestern Mesoregion and information on the number of people trained by municipality in the technique of artificial insemination (AI) was obtained from the Federal Institute of Education, Science and Technology of Rio Grande do Sul. The farmers (195) belonging to 216 municipalities that make up this mesoregion were randomly selected for analysis and classified according to production activities, capital investment, technology and use of artificial insemination. The variables were standardized, assuming mean zero and variance one. Analysis of variance was used to evaluate herd size

milk production and the size of the property. The variables used in the analysis were number of dairy cows, dry cows, calves up to 1 year, males, bulls, working animals, total number of animals, amount of milk produced per lactating cow, monthly milk production, price received per liter of milk and area for pasture production. Means were adjusted by least squares method. Qualitative variables (nutrition of dairy cattle, management of animals and pastures, capital investment, and reproductive technologies) were categorized. Multivariate analyzes including cluster, canonical discriminant and correspondence analyses were performed to determine the factors that could influence the sources of variation. The area of the property was set to 1 (up to 40 ha), 2 (between 40 and 100 ha) or 3 (over 100 ha) and the time in the dairy business as 1 (under 5 years), 2 (between 5 and 10 years) or 3 (over 10 years).

Results: Four clusters were formed due to production traits and farm size. The difference in properties of high production and large size area to low production and small size area was that the former had on average 11 times more total animals and lactating cows per year. They also produced monthly 20 times more milk and had assigned 44 times higher area for food production for the dairy herd. Two factors explained 69.76% of the variance between the clusters. Discriminant analysis showed that production best explains the difference among the clusters formed. Correspondence analyzes showed that more artificial insemination use is associated with higher milk production and factors related to production. Use of artificial insemination was linked to higher productivity, the size of the property, the length of time the farmer was in the dairy business and the higher price received per liter of milk sold. However a negative association among the use of AI and grazing native pasture, use of common salt and crop residues, and positive association with the use of conserved forage, concentrates and mineral salt for feeding livestock were observed. Also, the properties belonging to the municipalities that had the highest number of people trained for the use of AI was associated with increased production, the largest investment in area and land, physical, institutional and human resources. The association of the use of AI with embryo transfer, fixed-time artificial insemination, pregnancy diagnosis and sexed semen was positive.

Conclusions: The use of artificial insemination in the Northwestern Rio-grandense Mesoregion was associated not only with increased productivity, but also with the use of other technologies necessary to meet the needs of global production systems and specialized technified milk production.

Reproduction: (Dairy)

P03-003-232

Benchmarking reproductive parameters dairy farms worldwide

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Objectives: To describe and benchmark reproductive parameters in 10 dairy farms located in Spain, Portugal, Estonia, Finland and Mexico to find opportunities to improve in the reproduction management performance.

Materials and Methods: Several reproductive parameters were collected from 10 dairy farms by veterinarians in different regions of the

world (cows range): Northwest Spain (56 – 83 cows), Portugal (42 – 530 cows), Estonia (157 – 2,152 cows), Finland (33 – 130 cows) and Mexico – La Laguna (450 – 680 cows).

Initially veterinarians were asked to collect 7 parameters: days of calving to first service interval (CFSI), percentage of conception at first service (FSC%), days of calving to conception interval (CCI), percentage of heat detections (HD%), average of calving number (CN), percentage of average conception (C%) and Days in Milk (DIM). However it was not possible in several countries because their different software systems for keeping records did not include some of the proposed ones (Estonia) and, instead, others were provided (Finland): Non return rate - 60 days- (NRR%), Calving Interval days (CI), number of IA per calving (IA/C).

Descriptive results are reported by second quartile (Q_2) or percentile 50th, which represents the median / 50% of the data and, range (data dispersion/variation): Median (minimum - maximum).

Results: Spanish, Portuguese and Astonish farms had a close CFSI: 77 (67 – 122), 74 (55 – 82) and 74 (69 – 85) respectively. However Spanish dairies had a big variation, similar to Mexico but within a low range of days: 66 (56 – 100). While Finland reported: 88 days (80 -105).

Astonish farms got a good FSC%: 47 (34 – 55) and a small variation across farms. Spanish and Mexican farms had similar values: 32 (18 – 50) and 31 (28 – 40) respectively. However, variation across Spanish farms was high, as it was in Portuguese farms: 37 (22 – 51). Finish farms recorded a NRR% of 56 (43 – 76).

Within Spanish farms there was a high variation on the CCI: 146 (112 – 189). However, within Portuguese, Astonish and Mexican farms there was a low variation: 115 (106 – 137), 117 (105 – 136) and 115 (110 – 120) respectively. Finish farms reported a CI of 408 days (385 – 440) and Astonish farms had 393 days (383 – 409).

HD% is a subjective record as it depends on farmer's collection. It varied as follow: Spain 59 (46 – 68), Portugal 50 (36 – 66) and Mexico 50 (55 – 65). While Finland recorded IA per calving: 2,1 (1,5 – 3,2).

CN was from high to low values as follow: Spain 2,7 (1,9 – 3,5), Portugal 2,3 (1,9 – 2,8), Finland 2,2 (1,7 – 3,2) and Mexico 1,2 (1 – 2).

Spain and Portugal had a close C%: 39 (25 – 42) and 38 (29 – 47) respectively. However Mexican farms were far from this performance: 29 (25 – 32).

DIM varied widely among farms: Portugal 172 (159 – 186), Spain 157 (148 – 222) and Mexico 150 (140 – 160).

Conclusions: Spanish and Portuguese farms seems to have similar reproduction management as well as between Finland and Astonish farms. However, Mexican reproductive parameters differ from European countries; in contrast, variation within La Laguna farms was small.

Benchmarking across farms could not be established because of data source (equations) variation as well as some missing values. Moreover, differences on cow breeds reproduction performance and farm management must be considered. Therefore only a unified worldwide software able to correct for several parameters could bring opportunities to improve among countries worldwide.

Comments: *We will include more countries on the poster presentation.

Reproduction: (Dairy)

P03-003-233

Follicular dynamics in postpartum dairy cows with abnormal resumption of ovarian cyclicity

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Objectives: It has recently been reported that more than half of lactating dairy cows show abnormal resumption of ovarian function after calving. There are two typical patterns of abnormal ovarian resumption: the delayed first ovulation (DOV) and the prolonged luteal phase (PLP). The follicular dynamics in these abnormal postpartum ovarian cycles are not yet known. This study aimed to describe the follicular dynamics in postpartum dairy cows with abnormal ovarian cyclicity.

Materials and Methods: Fourteen cows that calved from Jul 2013 to Sep 2014 were used. Examination period for each animal was from 60 days before calving to the day of first artificial insemination after calving. Body weight, body condition scoring (BCS) and blood components (glucose, nonesterified fatty acid, 3-hydroxybutyric acid and γ -glutamyl transpeptidase) were analyzed to reveal nutritional state. Ovarian resumption and uterus involution after parturition were examined 3 times/week until insemination. Number and diameter of follicles and corpus luteum (CL) were diagnosed with ultrasonic echography. Follicles with diameter ≥ 8 mm were defined as dominant follicles (DF). Plasma progesterone (P4) concentration was measured by ELISA to monitor CL function for the same period. Pattern of ovarian resumption was classified as normal, DOV (first ovulation delayed >35 days after calving) or PLP (luteal phase prolonged >20 days); PLP was classified according whether prolonged luteal phase occurred at the first (PLPI) or second (PLPII) estrous cycle. The maximum diameter, frequency of ovarian domination, interval and duration of each follicular wave and number of ovulations were recorded to describe follicle development. Estrous signs were detected from genital changes and estrous mucus discharge, and also heat detection aids.

Results: After calving, 50% of the 14 cows had normal ovarian resumption, 28.6% of cows had DOV, 14.3% of them had PLP and 7.1% were others. All had normal uterine resumption. BCS change in the DOV and normal groups were similar. Ranges of BCS change and body weight loss after calving in PLP animals were slightly narrower than other groups. Blood glucose levels were higher in the DOV group than the normal group; other blood parameters did not significantly differ within groups. In the normal group (n=7), follicular waves occurred 1.5 ± 0.2 and 2.0 ± 0.0 times (in average \pm SD) with duration 6.3 ± 1.9 and 8.2 ± 1.2 days at the first and second estrous cycles, respectively. Concentration of blood P4 was 2.1 ± 0.2 and 2.8 ± 0.3 ng/ml at the first and second luteal phase. In DOV (n=4), 2.8 ± 0.5 follicular waves occurred with first ovulation 36.0 ± 0.0 days after calving. Duration of the first and second follicular waves after calving was 17.0 ± 3.3 and 10.0 ± 1.0 days. Follicular waves occurred 1.8 ± 0.3 and 2.0 ± 0.0 times with duration 7.3 ± 2.1 and 5.5 ± 1.2 days at the following cycles. Concentration of blood P4 was 2.1 ± 0.4 and 2.6 ± 0.4 ng/ml at the first and second luteal phase. In PLPI (n=1), follicular waves occurred 3 times with duration 15, 10 and 8 days in the prolonged luteal phase. The average blood P4 level was 3.0 ng/ml and diameter of CL was 27.3 mm in the same period. In PLPII (n=1), follicular waves occurred 4 times with duration 14, 10, 13 and 2 days in the prolonged luteal phase, with double ovulation. Average concentration of P4 in that phase was 3.1 ng/ml and diameter of CL was 20.2 and 22.4 mm.

Conclusions: Abnormal ovarian resumption in cows postpartum was similar with past reports. Follicular dynamics in DOV of relatively

longer duration of follicular waves was seen before first ovulation, but it normalized after recovery of estrous cycles. In PLPI and PLPII, relatively prolonged duration of follicular waves repeated within the prolonged luteal phases.

Reproduction: (Dairy)

P03-003-234

100µg gonadorelin acetate combined with cloprostenol sodium in a fixed-time artificial insemination program for estrous synchronization in dairy cows

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Objectives: In 2013, the use of 100µg of gonadorelin base (as gonadorelin acetate, GonaBREED[®]) was approved in the USA for sequential use with 500 µg of cloprostenol for use in single fixed-time artificial insemination (FTAI) programs (Ovsynch) in dairy cattle. The objective of this study was to generate clinical data to obtain US registration of a lower dose of gonadorelin (100µg of gonadorelin acetate, containing 86 µg of gonadorelin base, FERTAGYL[®]) for use in sequence with 500 µg of cloprostenol in the afore mentioned single fixed-time artificial insemination (FTAI) programs.

Materials and Methods: A clinical field study was conducted across seven dairy farms in six US states. Healthy non-pregnant dairy cows between 51-119 days in milk were randomized within farms to a gonadorelin-treated group (2mL Fertagyl[®], containing 86µg gonadorelin, present as the acetate salt) or a saline-treated control group. Enrolled cows were treated on Day 0 with 2mL Fertagyl[®] (N = 377) or 2mL saline (N = 381); Day 7 with 2mL Estrumate[®] (500µg cloprostenol); and on Day 9 with 2mL Fertagyl[®] or 2mL saline (as on Day 0). Cows were then bred by FTAI on Day 10, 16±8 hours after the Day 9 injections. Pregnancy checks were performed at least 30 days later. Most cows were first or second parity (gonadorelin group 62.1%; control 64.6%). Across the study, heat stress conditions were recorded on two farms: for 10 days on one; 2 on the other. Of 758 cows enrolled, 744 were included in the efficacy analysis.

Results: Overall, the pregnancy rate (PR) was significantly higher in the gonadorelin group compared to the control group (33.4% (125/374) vs. 17.8% (66/370); p=0.0051). There were no treatment related adverse events. Heat stress did not appear to affect the ability of the gonadorelin-cloprostenol synchronization treatment to increase PR, compared to the control group. The 33.4% PR obtained herein with a 86µg dose of gonadorelin (present as the acetate salt) compares favorably to the 33.4% PR reported for the 100µg dose of gonadorelin (present as the acetate salt) that was recently registered in the USA, and also with the 32.2% PR that has been reported in an Ovsynch FTAI program using a 200 µg dose of gonadorelin present as the hydrochloride salt.

Conclusions: The results of this study demonstrate that a 100µg dose of gonadorelin acetate (containing 86µg of gonadorelin base) used in sequence with 500µg cloprostenol is effective for estrus synchronization of dairy cattle in FTAI programs. The effectiveness of the lower dose of gonadorelin (86µg) used in this study compares favorably with that reported for higher doses of gonadorelin, whether present as the acetate or the hydrochloride salt.

Reproduction: (Dairy)

P03-003-235

Using inter-service intervals to monitor oestrus detection: does one size fit all?

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Objectives: Effective oestrus detection is essential in the modern dairy herd using artificial insemination. One frequently used method for evaluating oestrus detection is to assess the distribution of inter-service intervals.

The aims of this study were to:

- Assess inter-service interval performance in a large number of herds to provide data for benchmarking and target setting
- Evaluate what constitutes a “normal” inter-service interval for a modern dairy cow
- Identify whether the “normal” interval within a herd affects our interpretation of inter-service interval profiles

Materials and Methods: Herd management records were collected from 468 UK dairy farms. After checks were carried out to ensure appropriate data quality data from 74,923 cows in 167 dairy herd remained. Intervals between subsequent inseminations (serves) in the same cow in the same lactation were categorised in relation to the expected normal oestrus interval of 18-24 days. The standard categories include: short irregular (2-17 days), short regular (18-24 days), long irregular (25-35 days), long regular (36-48 days) and extended (49 days plus). Inter-service intervals profiles were calculated for every calendar year of every herd and ranked by oestrus detection efficiency to allow target setting. More detailed analysis of the distribution of inter-service intervals around the expected 21 day peak was conducted using multi-level regression modelling to identify cow factors affecting inter-service interval (Remnant et al, Journal of Dairy Science, 98 p.889). Data on this “normal” inter-service interval was then used to identify whether the interpretation of many herd inter-service interval profiles was affected by variations in the normal inter-service interval of a cow in the herd.

Results: The average performance of the top quartile of herd calendar-years was to have 6%, 40%, 16%, 19% and 19% of inter-service intervals in the short irregular, short regular, long irregular, long regular and extended categories respectively. Analysis of the inter-service intervals around the expected peak at 21 days revealed a modal interval of 22 days with significant associations between inter-service interval and parity, milk yield, service number and days in milk. However most of the variation in inter-service interval was between oestrus cycles within a cow and remained unexplained. This extended “normal” interval appears to affect the interpretation of oestrus detection data in some herds. No clear associations of herd-level factors with the effect on data interpretation were identified.

Conclusions:

- Inter-service interval profile performance of UK dairy herds appears to be considerably lower than traditionally quoted targets. This study suggests more appropriate and achievable targets as described by the top quartile of herd calendar-years described above.
- The normal inter-service interval of dairy cows appears to be longer than the traditional 18-24 days, we believe this to be a reflection of cows having a longer oestrus cycle than commonly accepted.
- This extended “normal” interval does affect interpretation of inter-service interval profiles, but it does not appear possible to identify which herds will be affected.

Reproduction: (Dairy)

P03-003-236

Influence of Bio-Active Peptides from Fpp (Fermented Potato Protein) on Reproductive and Production Performance in Dairy Cows

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Objectives: In dairy cows, there is evidence to link a NEB associated decline in fertility to reduced concentrations of Insulin like Growth factor I (IGF-I). IGF-1 is a likely candidate to mediate this effect of NEB on reproduction¹. Former research demonstrated that IGF-1 is involved in ovarian physiology and folliculogenesis^{2,3,4,9} and milk production in cattle⁵. Own research demonstrated a positive effect of Lianol[®] Dairy, a complementary feed based on highly digestible fermented potato protein, on IGF-1 levels in cows⁶. This study focuses on the effect of supplementing this new product on reproductive parameters and milk production in dairy cattle.

Materials and Methods:

Experimental animals

The experiment was conducted at Shen Xing dairy farm of Shanghai Bright Holstein Co. Ltd. Forty cows in close-up period were chosen based on parity, body weight (600±30kg), body condition scoring, and were assigned randomly into 2 treatment groups with 20 cows per group.

Experimental treatments

A control group was fed a basal diet; the treatment group was fed the same basal diet with addition of Lianol Dairy (25g/d/cow) from 20 days before calving to 40 days after calving.

The basal diet was formulated based on “Feeding standard of China dairy cattle”(NY/T 34-2004). The trial was conducted with 10 days adaptation and 170 days test period (from 20 days before calving till 150 days after calving or 150 days in milk (DIM). Both groups were fed with a total mixed ration (TMR) three times per day, with free access to water. The experiment duration was from Mar 20th to Nov 30th in 2014.

Measurements

Milk production of each cow was recorded at 7, 40, 60, 90, 120, 150 DIM during the test period. Estrus time and calculated conception rate at 90 d, 120 d and 150 d after calving. Conception was checked with SPB (pregnancy specific protein B) in blood of cows with ELISA. From interval calving-conception, the calving interval was calculated.

Statistical analysis

Data were sorted using Excel 2003 software and analyzed using SPSS16.0 by T test. The data of the conception rate at 120 d, and 150 d after calving were analyzed by chi-square test. Treatment effects were considered significant at P<0.05.

Results: There were no significant difference in average milk yield recorded. But treatment group had numerically higher milk yields than the control group (see table 1.). Especially for 7, 40 and 90 DIM with respectively 2.6 kg (+8%), 2.4 kg (+5.9%) and 2.7 kg (+7.4 %). Over the whole testing period, the daily milk yield was increased by 3.9 % (150 days).

Table 1. Effect of lianol Dairy on milk yield (kg) at different sampling points.

Sample point	Control group (in kg milk)	Liano [®] group (in kg milk)	P-value
7 DIM	32.4 ± 7.0	35.0 ± 8.5	0.301
40 DIM	40.8 ± 5.1	43.2 ± 8.1	0.256
60 DIM	40.6 ± 5.1	41.1 ± 7.0	0.827
90 DIM	36.1 ± 6.4	38.8 ± 6.0	0.253
120 DIM	35.4 ± 5.1	36.2 ± 4.4	0.650
150 DIM	32.4 ± 8.2	33.5 ± 7.1	0.697
Total period	36.1 ± 4.1	37.5 ± 5.6	0.440

There were no significant differences in interval calving-1st service and calving-interval between the two groups (Table 2). The average calving interval of the treatment group was shorter than this of the control group (380 d vs 397 d, p=0.118). The conception rate at 120 days and 150 days DIM was increased in the treatment group (resp. 80.0% vs 53.3%; P=0.121 and 93.3% vs 73.3%; P= 0.142).

Table 2. The effect of Lianol dairy on reproductive parameters: interval calving-1st insemination, calving interval and conception rate at different DIM.

Control group	Lianol group	P-value	
Interval Calving-1 st service (days)	83 ± 20	86 ± 13	0.578
Calving interval (days)	397 ± 35	380 ± 22	0.118
Conception rate (%):			
90 DIM	33.3 %	40.0 %	0.705
120 DIM	53.3 %	80.0 %	0.121
150 DIM	73.3 %	93.3 %	0.142

Conclusions: IGF-1 concentrations can influence follicle development during and after lactation directly by increasing the follicular response to gonadotropins or indirectly by acting on the hypothalamus and thereby influencing pulsatile LH release by the pituitary^{7,8}. Former research has demonstrated a positive effect of Liano[®] Dairy on plasma IGF-1 levels in cows⁶. This trial demonstrated a large effect on milk yield and reproductive performance. Supplementing the new product resulted in an increased milk yield production (+3.8%) over the whole trial period. The calving interval was reduced with 17 days and the conception rate was increased with 20%.

Reproduction: (Dairy)

P03-003-237

Ultrasound characteristics of ovarian cysts in cows – results of two-year observation

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Objectives: Ovarian cysts are still a significant cause of cows infertility. The ultrasounds examination provides opportunities for a more accurate diagnosis. The aim of the study was the morphological characterization of ovarian cysts in Polish Holstein Friesians.

Materials and Methods: The study included 270 cows and heifers in which, with the use of ultrasounds (iSkand, 7.5 MHz linearprobe), cysts were found. All of the animals came from 6 herds with milk production > 10000 liters. In all those herds regular veterinary examinations were taking place every 3-4 weeks. Ovarian structures with the cavity > 20mm in diameter and a luteinized wall > 3mm in diameter were recognized as lutein cysts, meanwhile structures with the cavity > 20mm and a luteinized wall ≤ 3mm were classified as follicular cysts (Hatvani, 2013).

Results: In general 323 cysts were evaluated. 220 (68%) in this group were follicular cysts, 103 of them (32%) were lutein cysts. The average diameter, surface area and volume were respectively 33mm, 897mm² and 22289 mm³. Cysts were observed mostly on the right ovary (74%), less often on the left (18%), or on both ovaries at the same time (8%). The average lutein cysts were bigger than follicular (34mm to 32,8mm). Symptoms of nymphomania appeared in 23 cows (8%), in 39% of those cows cysts were on both ovaries. There was no relation between size of cysts and appearance of nymphomania. The rest 267 cows were reported due to anestrus. The most common cysts were single-chambered, however there were also two-, three- or multi-chambered cysts (222, 39, 3 and 2 (68.7, 12.1, 0.9 and 0.6%, respectively). In rare cases ovarian cysts were accompanied by the corpus luteum (4%). Of all analyzed cysts in the period to 60 days after calving follicular creations occurred more often than lutein (57 to 43%). In the period from 60 day to 120 and > 120 day the incidence of follicular cysts was higher (resp. 74 and 72%). The percentage of follicular cysts in heifers, primiparous cows and cows after 2, 3 and 4 lactation and > 4 lactation was 6, 27, 36, 12, 10 and 9%, respectively. In 5,5% of cows, cysts persist intact despite of treatment.

Conclusions: Ultrasounds examination provides more valuable data, including the size, structure and types of ovarian cysts. It allows also to specify the connection between frequency of cysts and number of lactations, days after calving and clinical signs. On the base of ultrasounds examination more precise treatment can be introduced.

Reproduction: (Dairy)

P04-004-170

Monitoring Bovine Viral Diarrhea and Infectious Bovine Rhinotracheitis infections in dairy cattle using precolostral sampling

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Objectives: The objective of the present study was to evaluate precolostral sampling as a tool to determine Bovine Viral Diarrhea and Infectious Bovine Rhinotracheitis status and infection in Québec dairy herds.

Materials and Methods: A convenience sample of 346 sera from 50 Québec dairy herds was used in the study. Out of them, 259 samples were taken between February 2005 and March 2008 by veterinarians and dairy producers who participated on a voluntary basis. The additional 87 samples originated from a previous study and were collected between August 1999 and October 2000 and were stored at -70 °C. At the moment of the visit, herd information (vaccination strategy adopted in the farm and veterinarian's perception of the farm's reproductive performance) and individual information (calving date, number of lactations, date of

vaccination and type of vaccine administered to the dam) were recorded. Cocygeal vein puncture using a vacutainer tube was used to draw blood. Antibody detection was achieved using 2 commercial IBR and BVD ELISA Kits. Sample processing and interpretation were performed according to the instructions of the manufacturer. In order to confirm that the samples were collected before colostrum intake, gamma-glutamyl transferase (GGT) activity was measured. As suggested by Paré et al., (1996), a sample with a GGT value < 50 U/L was considered to be precolostral.

Descriptive statistics were used to determine the distribution of the samples by farm, vaccination strategy and type of vaccine administered to the dams. The association between the IBR and BVD calf status and the type of vaccine given to the dam was analyzed using the Fisher's exact test. Results were considered to be statistically significant if P<0.05.

Results: A total of 304 samples were confirmed to be precolostral (PS). Out of them, 259 were collected from calves born from vaccinated cows (VC): 176 cows with an inactivated (K) vaccine and 83 cows with a modified live virus (MLV) vaccine. Overall, more positive results to either IBR (OR=86; 95% CI: 17.8 to 415.7) or BVD (OR=199.3; 95% CI: 41.7 to 952.3) were obtained within the non-PS than within the PS. Within the PS, 7 samples were positive either to IBR or BVD or both. One sample was positive to both IBR and BVD, 2 samples were positive only to IBR, and 4 samples were positive only to BVD. All positive PS were collected from calves born from VC, while positive non-PS originated mostly (60%) from calves born from non-VC. Five samples were collected from calves born from cows vaccinated with an inactivated (K) vaccine while 2 samples were collected from calves born from dams vaccinated with a modified live virus (MLV) vaccine.

Among the non-PS, 11 samples were positive to either IBR or BVD or both. Five samples were positive to both IBR and BVD, 1 sample was positive only to IBR and 5 samples were positive only to BVD. Seven samples were collected from calves born from non-vaccinated cows while the remaining 4 samples were collected from calves born from vaccinated cows (3 cows with a MLV vaccine and 1 cow with a K vaccine).

Conclusions: Positive results among PS indicate calf exposition to the viruses in utero. Positive results among non-PS reflect the presence of antibodies against both viruses in the dams. When samples are collected from calves born from non-VC, one could hypothesize that viruses are circulating in the herd. Precolostral sampling is a practical monitoring tool to determine and monitor IBR and BVD herd status in field conditions. Confirmation of precolostral samples by GGT activity level provides additional confidence in this strategy.

Reproduction: (Dairy)

P04-004-171

Effects of Insemination Number and Days Postpartum on the Probability of Pregnancy at Insemination on Dairy Herds in the United Kingdom

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Objectives: Dairy herd fertility management is under increasing scrutiny in the European Union. Some conventional milk buyers are stipulating strategies that require selection of a subfertile population of cows before

hormonal intervention begins. Under management practices common in dairy herds in the United Kingdom, this study was designed to investigate how insemination number and days postpartum affect the probability of pregnancy at any given insemination, both at the cow and herd levels.

Materials and Methods: Milk recording data from National Milk Records (Wiltshire, UK) or the Cattle Information Service (Hertfordshire, UK) were collected from 240 dairy herds across the United Kingdom that had enrolled in a herd performance and monitoring scheme. Inseminations between 1st January 2012 and 31st December 2014 were included in analysis alongside corresponding testday milk records. Data were collated in DairyComp 305 (Valley Agricultural Software, USA) before being exported to Stata 13 (Statacorp, USA). Data were cleaned to exclude periods during lactations where the reliability of outcome or insemination data may bias the observed probability of pregnancy. A multilevel logistic model with a repeated measures structure (insemination within cow within herd) was fitted using a Markov-chain Monte Carlo approach with uniform priors (MLwiN 2.34; Centre for Multilevel Modelling, University of Bristol). The final model included insemination number, days postpartum, interservice interval, milk yield, calendar year, lactation group, linear somatic cell score (LSCOR) and four harmonic regression covariates to account for seasonality. Point estimates and ranges for average marginal effects of combinations of variables were obtained by simulation from the posterior distribution.

Results: The cleaned dataset contained 248,488 inseminations from 103,547 lactations in 66,388 cows on 228 herds, with 2,743 8th inseminations. Median herd 305d milk yield was 9145kg (IQR: 8316-9890kg), with mean conception rates for 1st lactation, 2nd lactation and >2nd lactation of 35.2% (95% CI: 34.9-35.5%), 31.8% (95% CI: 31.5-32.2%) and 29.1% (95% CI: 28.9-29.4%) respectively. At the cow level, controlling for other variables, odds ratios for inseminations 2 to 5 were higher than the reference 1st insemination ($P < 0.001$), with a peak of 1.14 (95% CI: 1.08-1.20) at 4th insemination. Odds ratios for the 6th to 8th inseminations were not different from the 1st ($P > 0.05$). Inseminations between 42 and 49 days postpartum (inclusive) had lower odds of pregnancy than the reference 50-70 day window ($P < 0.001$), corresponding to a 5.3% (95% CI: 3.9-6.7%) absolute difference in probability of pregnancy, assuming a baseline of 35%. The pattern of interservice intervals changed by both days postpartum and insemination number, emphasising their confounding effect in this study. At the herd level, observed cow-level residual error in the eligible population changed by both insemination number and days postpartum. Accounting for these changes there was a downward trend in probability of pregnancy by insemination number, but only the 7th and 8th inseminations were significantly different from the first ($P < 0.05$). There were no significant changes in odds of pregnancy by days postpartum once the combined effects of milk yield, lactation group, LSCOR and the eligible population were considered.

Conclusions: The use of days postpartum or insemination number to identify dairy cows with a low probability of pregnancy at the next insemination was not supported by this study. For similar herds to those investigated, herd fertility protocols would appear to be misguided if they rely on self-selection of a subfertile population before hormonal intervention starts. However, this study did not investigate variation in the probability of insemination. Reduced probability of pregnancy for inseminations before 50 days should not be taken in isolation, and may suggest room for improvements in transition management. Analysis continues at the time of writing.

Comments: This study was pursued in partial fulfillment of an Alternative Residency with the European College of Bovine Health Management.

Reproduction: (Dairy)

P04-004-172

Detection of Under-Recorded Inseminations, Poor Oestrus Detection Specificity, or Abnormally Low Conception Risks in Multiherd Datasets

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Objectives: Under-recording of unsuccessful inseminations leads to a bias in the observed conception risk towards lower insemination numbers. At the extreme, a 100% conception risk would be observed for first insemination. This bias may occur through poor data collection or by use of a stock bull. Abnormally low oestrus detection specificity would bias probability of pregnancy towards higher insemination numbers. As part of a study investigating conception risks, a graphical outlier detection technique was used to identify herd-years where insemination data was unreliable.

Materials and Methods: Milk recording data from National Milk Records (Wiltshire, UK) and the Cattle Information Service (Hertfordshire, UK) were collected from 240 dairy herds across the United Kingdom that had enrolled in a herd performance and monitoring scheme. Inseminations between 1st January 2012 and 31st December 2014 were included in analysis alongside corresponding testday milk records. Data were collated in DairyComp 305 (Valley Agricultural Software, USA). Lactations were right-censored to exclude periods during lactations where data reliability of recorded inseminations may bias the observed probability of pregnancy. Using non-censored inseminations, annual average 21-d Insemination Risk (21-d IR), 21-d Pregnancy Risk (21-d PR) and Conception Risk (CR) were calculated for each calendar year in each herd (herd-year) using a standardised 50 day voluntary waiting period.

To detect herd-years for which insemination data may be missing or unreliable, robust multivariate outlier detection was performed in R 3.2.0 (R Core Team 2015) using an iterated reweighted minimum covariance determinant procedure. Herd-years were marked as outliers if apparent relationships between annual averages for 21-d PR, 21-d IR and CR were unlikely for the dataset ($p < 0.025$), after approximating the distribution of their robust Mahalanobis Distances with an F distribution. The three-dimensional distribution of all herd-years was examined graphically. Observed conception risks were compared with those calculated after exclusion of data affected by outlying herd-years.

Results: Before outlier detection the cleaned dataset contained 269,992 inseminations between 1st January 2012 and 31st December 2014 from 240 herds. These data were clustered in 688 herd-years, of which 56 were identified as outliers by multivariate outlier detection, although only six herds were identified as outliers in more than one year.

Two main clusters of outlying herd-years were identified; Given their respective 21-d PR, one group had high mean CR and low 21-d IR. This pattern would be expected if a herd were systematically under-recording inseminations, or used a stock bull extensively. A second group of outliers had low mean CR and high 21-d IR when compared with their 21-d PR. This might suggest that oestrus detection specificity was low, pregnancy diagnosis results were not reliably recorded, or another process was reducing conception risks at the herd level. For this group of outliers, under-recording of pregnancy diagnosis results could be excluded by matching inseminations to results. Robust multivariate outlier detection excluded biologically implausible mean conception risks and identified other problems in the dataset. The potential bias in conception risk by insemination number induced by outlier censoring was minimal after the first insemination.

Conclusions: Robust Multivariate outlier detection using 21-d PR, 21-d IR and CR has potential to assist epidemiological studies into factors affecting conception risk. Furthermore, it may provide valuable information to assist large animal practitioners both in troubleshooting herd fertility problems and identifying herds with unreliable data. However, calculations for 21-d PR and 21-d IR in this study excluded inseminations censored in initial data cleaning in order to maintain the relationship between these variables and CR, so absolute values for 21-d PR and 21-d IR do not reflect true herd performance. Analysis is ongoing at the time of writing.

Comments: This study was pursued in partial fulfillment of an Alternative Residency with the European College of Bovine Health Management.

Reproduction: (Dairy)

P04-004-173

Incidence of false positive oestrus in pasture-based, seasonally bred cows

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Objectives: In the seasonal breeding system employed within the dairy industry of New Zealand, there is a strong emphasis on maximising sensitivity of oestrus detection. However, where false positive diagnosis of oestrus occurs conception will not occur, and in the case where pregnancy is already established, there is a high risk of embryo loss. Some studies have reported false positive oestrus rates of greater than 30%. Hence the objective of this study was to determine the incidence of false positive oestrus events during the period of artificial insemination.

Materials and Methods: Cows (n=556) from 15 herds (average herd size = 388 (SE= 277) range = 130 – 290) were enrolled. Herds were visited on 2 to 5 occasions coincident with the visit of the artificial insemination technician. All cows presented for AI that day were enrolled. These visits occurred between the 2nd and 4th week of the AI periods for the herds. Milk samples (2 to 4ml from a single gland) were collected into a vial containing a preservative (13mg of potassium dichromate in 200µl). The progesterone concentration was determined using an ELISA (Ridgeway Scientific, Gloucestershire, UK). A false positive oestrus was defined as occurring where the progesterone concentration was >2.5ng/mL. The artificial insemination technician was also asked to score the ease of insemination and the degree of mucus on an ordinal scale. The data were described, graphed and the exact 95% confidence intervals were calculated (Stata version 13.0)

Results: The mean milk progesterone concentration was 0.5 (SD=0.91) ng/mL. A total of 13 of the 556 (2.3% (exact 95%CI=1.3-4.0%)) cows had milk progesterone >2.5ng/mL indicating a likely false positive oestrus detection event. Nine of the 15 (60%) herds had no false positive cows, although due to the small sample size in some herds the confidence around these estimates is wide. The within herd prevalence of false positives ranged from 0 (exact 95%CI=0-13.7%) to 10.7% (exact 95%CI=2.3-28.2%). Cows presented for a second insemination were nearly four times more likely to be a false positive oestrus than those presented for their first insemination (5/68 (7.3%) vs 4/212 (1.9%) for 2nd vs. 1st service, respectively; relative risk=3.9, P=0.03).

Conclusions: The prevalence of false positive oestrus events was less than 3%. However, false positive oestrus does not appear to be a major

risk factor for poor reproductive performance in the seasonal breeding system practised in New Zealand. The prevalence of false positive oestrus was higher in the second three weeks of the seasonal breeding program, than the first. The consequences of a false positive oestrus in the second 3 weeks, when a proportion of cows are likely to be pregnant, is likely greater than where false positive oestrus event and insemination occurred in the first 3 weeks when few cows were pregnant.

Reproduction: (Dairy)

P04-004-174

Maternal heart rate dynamics during the second stage of labour in dairy cows

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Objectives: Determining the onset of calving and monitoring the progress of labour is a challenging and time consuming process. A peripartum around-the-clock observation is regarded as the best method to detect dystocia in time. As a result of individual differences in behaviour, a practical tool which can be easily used on farms with sufficient accuracy is still missing. Therefore, the purpose of this study was to collect data of heart rate variability (HRV) on the basis of interbeat interval (IBI) measurements during parturition, in order to identify characteristic patterns for the onset of labour and calving process in dairy cows.

Materials and Methods: The study was performed at the Teaching and Research Farm Kremesberg, University of Veterinary Medicine Vienna, Austria. For this study, the cows (n=31) were moved from the group pen to a single calving box approximately 6 days before the expected calving. The straw bedded boxes were equipped with a 24h operating digital video recording system. In addition, interbeat intervals were measured using a Polar Equine H3 heart rate sensor and a RS800CX watch (Polar Electro Oy, Helsinki, Finland). In order to observe the dam's behaviour and posture, the video recordings were retrospectively analysed from 1.5 hours antepartum until the expulsion of the calf. The main recorded events were: lying, standing and abdominal contractions during lying bouts. Initial fragmentary bouts were counted as a completed period. Coherent abdominal contractions (n≥3) were classified as visible signs of labour. In a first step, the IBI measurements have been displayed graphically and were observed for characteristic patterns. In a second step, these findings were matched to the video recordings to determine causalities. Finally, the heart rate events were compared to the classified videos used as the gold standard.

Results: Overall, 20 IBI data sets were recorded in an evaluable quality. In 19 data sets, visually detectable patterns were found, shown as periodically appearing decelerations. Within these phases, the heart rate graph displayed v-shaped oscillations, varying in number, frequency and amplitude of the spikes. The decelerations appeared simultaneously to active phases of expulsion and every single spike occurred during an abdominal contraction. While evaluating the heart rate events, the highest correlation was found between two encoded events: visible signs of labour, and appearing spikes, both being present during the last lying bout before the expulsion of the calf. It can be assumed that the decelerations occurred during uterine contractions and, moreover, the amplitude of the spikes were related with the intensity of the

labour, which can be explained by the Frank-Starling mechanism and sympathetic activation.

Conclusions: The preliminary results of this study illustrate the potential of the use of the maternal heart rate as a peripartum monitoring parameter. Further data analyses and research is needed to prove these findings and to evaluate a new peripartum supervising system. A real-time heart rate based monitoring program could reduce calf mortality by providing an optimized calving management.

Reproduction: (Dairy)

P04-004-175

Therapeutic Evaluation of a Treatment Regimen in Dairy Cows with Acute Puerperal Metritis and Post Treatment Reproductive Performance

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Objectives: Acute puerperal metritis (APM) is defined as a condition with an abnormally enlarged uterus and fetid, watery, reddish-brown uterine discharge, associated with systemic illness and fever within three weeks of calving. It is one of the major problem faced by dairy enterprises. Incidence rate ranges from 20-40%. Present study was conducted on a commercial dairy farm in from January, 2014-June, 2014. The objectives of this field study were to assess the diagnosis and a treatment regimen in dairy cow affected with APM and subsequent reproductive performance post treatment.

Materials and Methods: Totally 578 cows were routinely examined from day 2-15 postpartum. Cows were monitored for appetite and behavior on daily basis. Vaginal discharge was examined by vaginal inspection and rectal examination on alternate days. Cows with rectal temperature >39.5 °C, a large uterus with a thin uterine wall, fetid, watery, reddish-brown abnormal uterine discharge, when examined vaginoscopically, and loss of appetite were considered as APM positive cases. Cows not determined abnormal vaginal discharge, loss of appetite, depression, and with rectal temperature < 39.2 °C were considered as normal (without APM). Thirty cows with APM were enrolled in treatment group. Thirty cows without APM considered as control group with no treatment. Considering the life-threatening character of APM, no untreated control group consisting of APM was included in this study. Cows once diagnosed for APM were subjected to a treatment regimen consisted of Ceftiofur@ 2.2 mg/kg BW for 5 consecutive days intramuscularly from day 4th to 8th pp, intrauterine Cephapirin (500 mg) and prostaglandin I/M (25mg) on 15 day postpartum. Therapeutic cure of cows with APM was defined as the absence of fetid, watery, reddish brown abnormal uterine discharge examined on vaginoscopy, and disappearance of loss of appetite, depression, and the decrease of rectal body temperature (< 39.2) on day 6 after first ceftiofur administration.

Results: Data were analyzed using the SPSS. Independent sample t-test for parametric assumptions and Mann-Whitney test for nonparametric data were used for comparisons between groups. Fisher's Exact and chi-square tests were performed to test the differences between groups of categorical data ($p < 0.05$). Continuous variables were indicated separately according to the groups as means \pm SD. Vaginal discharge scores (VDS) for treatment (3.37 \pm 0.49, 2.37 \pm 0.93, 1.60 \pm 1.07, 0.37 \pm 0.67, 0.20 \pm 0.20) and control groups (1.40 \pm 1.13, 1.00 \pm 1.0, 0.27 \pm 0.64, 0.13 \pm 0.43) cows at different visit days (from V1 to V5) were calculated. Cervical and Uterine sizes (cm) for treatment and control groups cows at different visit

days. On visit V1 and V2 cervical diameter was 8.26 \pm 1.20 and 6.43 \pm 0.85 with corresponding control group having 4.87 \pm 0.77. Uterine horn (UH) diameter was 13.42 \pm 2.32 vs 12.51 \pm 1.03 in treatment and control group on V2 respectively. On Visit V5 uterine horn diameter was 5.76 \pm 0.61 in treatment groups as compared to 2.88 \pm 0.24 in positive control group. PMNs percentages on V2 were significantly higher in treatment group (48.17 \pm 10.07) as compared to control group (31.73 \pm 9.70). On V5 PMNs percentage was 4.10 \pm 5.74 in treatment group which was comparable with control one (4.67 \pm 6.17). First service conception rate was 40% and 43% in treatment and control group respectively. Pregnancy rate (87%) was comparable in treatment group with control one (90%) after overall inseminations.

Conclusions: Results of present study prove that systemic and intrauterine antibacterial therapy combined with prostaglandin has beneficial impact on reproductive performance in dairy cattle with acute puerperal metritis.

Reproduction: (Dairy)

P04-004-176

Evaluation of the effect of trace elements and vitamins boli on fertility parameters in dairy cows.

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Objectives: The costs of infertility can be very high for the farmer both in direct costs, like the multiplication of the number of artificial inseminations, as the hidden costs, more difficult to assess, such as loss of milk production or increase of the reform ratio due to infertility. The objective of this clinical trial was to evaluate the impact of a Vetalis bolus based on 5 trace elements (Co,Zn,Mn,I,Se), provitamins and vitamins (A,D3,E and beta-carotene), on the fecundity, particularly on the fertility of the dairy cattle. Beta carotene is an essential pre-cursor of vitamin A in the synthesis of progesterone from cholesterol by the corpus luteum.

Materials and Methods: 92 milk cows of the Prim Holstein race mainly, spread over 3 different farms with a different technical level have received a bolus on the day of calving in order to prepare the reproduction period. Group A, 49 cows, have received a placebo bolus, Group B, 43 cows have received a Vetalis bolus. Three parameters were investigated: interval calving - successful artificial insemination (parameter of fecundity), interval calving - 1st artificial insemination AI1, expressed in days and number of artificial inseminations (parameter of fertility). The products has been administrated under the supervision of the responsible veterinarian. A method of random alternating selection has been used to put up the different animal groups A and B.

Results:

- Study of the interval calving-fecundity insemination parameter (IC-FI)

On the IC-FI interval, we find a statistically significant herd effect, meaning a significant difference in between the herds X, Y and Z (Two factor ANOVA method- treatment and farm). Although no significant difference between the two groups A & B, neither in between the treatment effect on the herd, we can conclude an improvement after the use of the Vetalis bolus in the group B. Indeed, the IC-FI interval is 11 days shorter in group B compared to group A. This trend is even more important in the herd X, with a difference of 25 days in favor for the group that has received the Vetalis bolus.

- Study of the interval calving–first artificial insemination (IC_AI1)

We haven't distinguished significant differences between the treated group and the control group of animals on this parameter. The supplementation with the Vetalis bolus seems not to have influences of the heat detection in this study.

- Parameter "Number of artificial inseminations"

On the number of AI we observe a significant difference in between the 2 groups in favor for the group that has received the Vetalis bolus. PS : When the data are adjusted with a generalised linear model, the treatment effect is close to the threshold of statistical significance ($p=0.05972$). This effect becomes significant when we use a generalised linear model with mixed effect, meaning fixed and aleatory ($p=0.0484$). The herd effect is also significant (generalised linear mixed-effect model), meaning the number of AI is depending on the farm.

Conclusions: The results show that a supplementation with trace elements, vitamins and beta carotene have a statistically significant action on fertility and a clear tendency to improve the fecundity. The improvement of the number of inseminations is an average gain of 0,323 inseminations, meaning for a reproduction cycle of 21 days (theoretically in between 18 and 24 days), a gain of 6,9 days for the all farms and more precisely 16,6 days for farm X.

The bolus formulation, based on trace elements (Se,Zn,Mn,Cu,I), vitamins (A,D3,E; b-carotene precursor)occurs effectively in improving the fertility, even in non-optimal prescription conditions.

Comments:**- Estimated costs of a delayed in calving-calving interval:**

For an interval calving-calving longer than 400 days, the cost per delayed day is estimated at 3€(Verdoolaege, PLM) This means that in average, the use of a Vetalis bolus, saves 20,40€ per cow or 1032€ per herd with an average of 50,6 cows. The average AI-FI found in group A is 127 days. Taking in consideration a gestationperiod of 282 days, the calving-calving interval is 409 days in total. In the herd X, the supplementation with the Vetalis bolus has an economical gain of 49,80€ per cow, meaning 2520€ for a herd with an average of 50,6 cows. The interval AI-FI found in the herd X is 169,28 days, whether 450 days of calving-calving interval.

The results show that a supplementation with trace elements, vitamins and beta carotene have a statistically significant action on fertility and a clear tendency to improve the fecundity. This supplementation seems not to have influences on the cycle and the heat detection. On the other hand, we have noticed an action on the persistence of the gestation. According to scientific literature, the presence of a sufficient amount of beta-carotene is needed to complete the Vitamin A which is essential for a good growth of the corpus luteum and the local progesterone synthesis to attain an optimal plasmatic concentration. The progesterone promotes the elongation of the embryo and increase of the signal strength to maintain the gestation by the foetus. Beta-carotene and vitamin A are primary in the maintenance of the gestation. Vitamine E as Selenium are limiting the oxidative stress especially around ovulation. The trace elements are mainly involved in the hormonal system: I and Se in the synthesis of the thyroid hormones, Co and Zn rather the pituitary level.

The chelate form enables a direct assimilation in the bovine intestine, with a faster valorisation and efficacy of the Co Zn and Mn cations. Acting in synergy, they provide a support on the progesterone production and an increase on the fertility. The herd effect is important and allows us to assume an interesting action on farms with marked reproduction disorder as we can find in the farm X. This should be investigated in a new clinical trial on equal farms as farm X. The bolus was administrated at calving which is not the ideal timing for supplementation if we want to interfere with the reproduction period. However it is a strategic moment in the « field farm ». Indeed, we can assume that trace elements will be used to level up low trace elements concentration to address the needs

for calving preparation, placental transfer to the foetus and synthesis of colostrum. They will be less available for use in the context to improve fertility. We could consider exploring the trace element statutes before bolus administration and after his lead time. The interest was to demonstrate significant action even under non-optimal conditions.

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Reproduction: (Dairy)

P04-004-177

Comparison of urine Pregnancy-associated Glycoproteins (PAG) and ultrasound as a specific method of late embryonic mortality diagnosis in dairy cows

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Objectives: The aim of this study was to determine whether pregnancy-associated glycoprotein (PAG) concentration in urine of pregnant cows is as specific marker of late embryonic mortality (LEM) as ultrasound. For this purpose combine measurements of concentration of PAG in urine and transrectal ultrasonographic examination of the conceptus were performed.

Materials and Methods: The analyzed data were derived from 36 multiparous Holstein Friesian cows (BCS 3,25-3,5). Animals were divided into 3 groups: pregnant (P, n=16), non-pregnant (NP, n=10) and cow diagnosed with late embryonic mortality (LEM, n=10). Transrectal ultrasonographic examination and urine samples were obtained from all animals on days: 0 (AI day), 14, 21, 28, 35, 49, 63, 77, 91 and 105. Radioimmunoassay (RIA -706) was performed to determine cPAG (caprine PAG) concentration in urine. During the time all animals underwent pregnancy testing with B-mode diagnostic ultrasound Scanner (Mindray DP – 6600) equipped with a 7.5 MHz linear-array rectal probe and rectal palpation at each time point established. The diagnosis

of pregnancy indicates the presence of allantoic sac and the embryo presence, and after day 35 the heartbeat of the embryo. Late embryonic mortality or fetal death was diagnosed when observed embryo/fetus showed no heartbeat, or when previously observed embryo was not visible during subsequent ultrasound.

Results: The study of PAG concentrations in urine demonstrate an increased concentration of these proteins (>1,5ng/ml) in group of non-pregnant cows and cows with LEM. Identified low concentrations during pregnancy (<1ng/ml) in group of pregnant cows did not correlate with the period of pregnancy duration and varied more between individuals. In the context of late embryonic mortality diagnosis, PAG urine test proved to be as high sensitive and specific method as ultrasound examination.

The data obtained indicate the possibility of using PAG research in the urine as predictor of LEM. Marked significant differences occur as early as 5 weeks after insemination pointing to the high sensitivity and specificity of the method comparable to ultrasound. The accuracy of both tests at this time point was similar: sensitivity 98,3% for urine PAG, 100 % for ultrasound, specificity 90% and 98% respectively. Only the method of urine PAG analysis were able to detect pregnancy at stage of 28 day of gestation. (sensitivity 98% for PAG urine, 68% for ultrasound and specificity 90% and 70% respectively). However, by the end of the study the accuracy for ultrasound examination was 100% and for urine PAG method ranged between 75-100%.

Conclusions: Based on the study it can be concluded that the concentration of the PAG in the urine is a valuable source of information on the developing embryo / fetus. PAG concentration in urine is a sensitive indicator of pregnancy losses and requires further research to better understand and to develop methods for early detection of late embryonic mortality. In the context of late embryonic mortality diagnosis, PAG urine test proved to be as high sensitive and specific method as ultrasound examination.

Reproduction: (Dairy)

P04-004-178

High level of estradiol in sacroiliac luxation Holstein-friesian cow after calving

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Objectives: Sacroiliac luxation was reported to occur rarely in cows during the periparturient period and high estrogen concentration was one of predispose through this phase. Prognosis is very poor because dystocia could not be avoided during calving period. The objective of the study is to serve the information to large animal practitioner that the case is rarely observed in the field.

Materials and Methods: A Holstein-Friesian cow was referred to animal clinic with unable to rise. The cow, on its fifth day after calving, had fallen on the slippery surface when it walked out from milking parlour after morning milking. It was moved to the soil ground by farm producers to prevent secondary injury due to the slippery floor and was crying in pain, with growling sound, for hours. Non steroid anti-inflammatory drug was injected into the cow to relieve the pain and antibiotic was restricted for preparation of the cow to be slaughtered.

Results: The next day, the cow was able to stand up after and regain its good appetite. The tuber coxa was quite raised above the lumbar vertebra and the gait posture of the hind limbs was rather unstable swing

back and forth. The estradiol(E2) level of the cow was high (average E2 concentration: 167 pg/mL) compared to those with normal standing posture (average E2 concentration: 41.4 pg/mL) within the same barn. The high E2 concentration continued for several days after the onset of the disease. Similarly, CK(creatine kinase), AST(aspartate aminotransterase) , LDH(lactate dehydrogenase) were also high in range while both the calcium and phosphorous levels were within the normal range as revealed by the blood chemistry analysis.

Conclusions: In conclusion, the cow finally was slaughtered due to the gait posture became more worsen though the cow had maintained good appetites. It would be presumed that the cow showed typical sacroiliac luxation after calving associated with the high level of E2 concentration.

Reproduction: (Dairy)

P04-004-179

Can in practice the success of insemination be predicted with a systematic assessment of the heat?

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Objectives: The success of insemination depends on several factors. It includes hosing of the bull, semen handling as well while the straw production as the insemination and the cow ready to conception. The quality of estrus of all cows inseminated by Swissgenetics SG will be evaluated with a simple system. Findings of an assessment of the cow at time of insemination are required to predict the probability of conception from the inseminated cow. The second aim of this assessments is to calculate the factor cow in the calculation of the nonreturnrate NNR of the bull and to estimate their influence in the work of the inseminator.

Materials and Methods: The records of all inseminations registered at SG from 07.01.2013 to 30.06.2014 were evaluated. The results of estrus assessments were compared to the NNR at day 56 post insemination. The following for criteria were assessed 1. information by the farmer: good heat, weak heat, Instruction by the veterinarian, no information. 2. rating of the point in time by the inseminator: in time, rather early, rather late, unclear signs . 3. findings of the sex organs: normal, large uterus, obscure mucus, urovagina, suspected cyst. 4. special findings: intravaginal device, other hormonal control, induced ovulation, other treatment by the veterinarian. Evaluations were made using excel.

Results: In the observation period total 835'281 inseminations were performed. 710'544 were performed by inseminators of SG and reported according to internal directives. Private veterinarians inseminated 124'736 cows. These are not required to assess the estrus; therefore from these there are no further information on estrus while insemination. The NNR 56 considering all inseminations was 69.78%, while the insemination success between inseminator (69.79%) and veterinarians (69.08%) was not significantly different. Were the heat qualitatively good and the time optimal assessed (610'566), the insemination success (70.93%) was significantly better than over all inseminations. The insemination success of a cow at earlier insemination in a good heat was not unfavorable affected (NRR 68.61 %). The other hand, a cow with an urovagina, their NRR dropped to 61.73 % although good estrus signs. Basically, a hormonal treatment for estrus induction reduces the NRR of less than 64%. However, showed the treated cows good estrus signs and the organs were assessed to be normal, the NRR (67.18%) of those cows were no longer differed from the average. Were findings assessed as different from the physiological properties, the NRR decreased: an enlarged uterus diminished the NRR to 60.42 % and a reduced NRR followed of changes in vaginal fluid (59.88 %).

Conclusions: The result shows, in practice a structured assessment of estrus signs before insemination is very useful. A possible success and especially failure of the insemination can be estimated. For a more differentiated expectation, the combination of different findings is important. Subsequently, calculation of the NRR, therefore the factor cow can be considered by systematic assessment of the heat. This more accurate calculation allows a differentiated evaluation of the quality of bull semen and the work of the inseminator.

Reproduction: (Dairy)

P04-004-180

Comparison of urine Pregnancy-associated Glycoproteins (PAG) and ultrasound as a specific method of late embryonic mortality diagnosis in dairy cows

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Objectives: The aim of this study was to determine whether pregnancy-associated glycoprotein (PAG) concentration in urine of pregnant cows is as specific marker of late embryonic mortality (LEM) as ultrasound. For this purpose combine measurements of concentration of PAG in urine and transrectal ultrasonographic examination of the conceptus were performed.

Materials and Methods: The analyzed data were derived from 36 multiparous Holstein Friesian cows (BCS 3,25-3.5). Animals were divided into 3 groups: pregnant (P, n=16), non-pregnant (NP, n=10) and cow diagnosed with late embryonic mortality (LEM, n=10). Signs of oestrus exhibition day was the "0" day. In P and LEM group AI was then conducted. Transrectal ultrasonographic examination and urine samples were obtained from all animals on days: 0 (AI day), 14, 21, 28, 35, 49, 63, 77, 91 and 105. Urine was obtained by catheterization. Blood was sampled from the tail vein (v. caudalis mediana). Samples has been deep-frozen and storage until analyses. RIA (RIA -706) was performed to determine cPAG (caprine PAG) concentration in urine. For the analysis of urine material, a modification of the method RIA III developed for the testing of PAG in milk with larger volumes of the test material was used. During the time all animals underwent pregnancy testing with B-mode diagnostic ultrasound Scanner (Mindray DP – 6600) equipped with a 7.5 MHz linear-array rectal probe and rectal palpation at each time point established. The diagnosis of pregnancy indicates the presence of allantoic sac and the embryo presence, and after day 35 the heartbeat of the embryo (Szenzi et al., 1998). Late embryonic mortality or fetal death was diagnosed when observed embryo/fetus showed no heartbeat, or when previously observed embryo was not visible during subsequent ultrasound.

Results: In contrast to the C group, the breakdown statistically different groups was not possible, the results of a group of LEM divided into two groups differ significantly depending on the time of the material collection: Group A (0 - 5 weeks after insemination), group B (7 - 11 weeks after insemination).

It was found no statistically significant differences between the PAG concentrations in the urine in study groups between 0 and 4 weeks after insemination. At 5, 7, 9 and 11 weeks after insemination we observed a statistically significant higher levels of PAG concentration in urine of

the cows from LEM group compared to P group. From 5 weeks after insemination it was observed directly proportional increase in the concentration of PAG in the urine of LEM cows until the end of the study. Cows diagnosed with late embryonic mortality at 5 weeks were higher than 1.5 ng / ml. The data obtained indicate the possibility of using PAG research in the urine as predictor of LEM. The accuracy of both tests at this time point was similar: sensitivity 98,3% for urine PAG, 100 % for ultrasound, specificity 90% and 98% respectively. Only the method of urine PAG analysis were able to detect pregnancy at stage of 28 day of gestation. (sensitivity 98% for PAG urine, 68% for ultrasound and specificity 90% and 70% respectively). However, by the end of the study the accuracy for ultrasound examination was 100% and for urine PAG method ranged between 75-100%.

Conclusions: Based on the study it can be concluded that the concentration of the PAG in the urine is a valuable source of information on the developing embryo / fetus. PAG concentration in urine is a sensitive indicator of pregnancy losses and requires further research to better understand and to develop methods for early detection of late embryonic mortality. In the context of late embryonic mortality diagnosis, PAG urine test proved to be as high sensitive and specific method as ultrasound examination.

Reproduction: (Dairy)

P04-004-181

Correlations between the volume of the corpus luteum and plasma concentration of progesterone in the estrous cycle of the cow.

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Objectives: The aim of present research was to evaluate the correlation between morphology and the volume of the corpus luteum with progesterone levels in the blood plasma of the reproductive cycle in dairy cows (Holstein-Friesian).

Materials and Methods: Ultrasound scans were carried out every 48 hours, using an ultrasound Sonovet 2000, with a multifrequency probe LV4-7AD designed to transrectal insertion. Several discriminative parameters of gestation (major and minor diameters of the corpus luteum, area and volume) were studied. In addition, progesterone levels in plasma, obtained of venous blood, was determined radioimmunologically using Spectria kits (Orion, Diagnostica, Finland). The limit of assay sensitivity was 94 pg/ml. The coefficients of variation within and between assays were 5.8 and 2.9% respectively. The mean recoveries were 95.4-104.2%

Results: Until the day 10 of the cycle, the luteal volume and concentration of plasma progesterone have been increasing. The growth rate of luteal volume is greater than the growth rate of the plasma progesterone in this period. However, from day 11 to 17 of the cycle, progesterone values which increases more sharply. Since the day 17, it begins to be sensitive process of luteal regression, down the values of luteal volume and plasma progesterone. The drop in progesterone levels is much faster and charged, especially between 20 and 21 drops to almost baseline levels.

Conclusions: It has been found that up to day 10 of the cycle, both the volume of the corpus luteum, as the concentration of plasmatic progesterone have increased. In this growth period, luteal volume is

greater than the growth plasmatic progesterone. From day 11 to 17 of the cycle, progesterone values increases more sharply. However, between 20 and 21 days, progesterone levels fall quickly, reaching baseline values.

Reproduction: (Dairy)

P04-004-182

Virulence factors profile and determination of phylogenetic group of *Escherichia coli* isolated from the uterus of Holstein cows in early postpartum

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Objectives: This study aimed to evaluate pathogenic potential of *E. coli* involved in the uterine contamination on first days postpartum of healthy cows by the identification of virulence factors and phylogenetic grouping.

Materials and Methods: Data were collected from a dairy farm located in Araras, Brazil, on 75 Holstein cows with a mean age of 2.9 years (± 1.4), between first to sixth parity order, with normal calving and physiological puerperium, free from treatments with antibiotic or anti-inflammatory drugs during the experimental period of 14 days. Samples of uterine contents were collected 24 hours (Moment 1) and 14 days postpartum (Moment 2) for bacteriological analysis. All samples were cultured in MacConkey agar in aerobic conditions at 37°C for 48 hours aiming the isolation of *E. coli*. This bacterial specie was confirmed by the biochemical profile on the tests of glucose fermentation, gas production, L-TD (L-tryptophan deaminase), H₂S, urease, Simmons citrate, lysine, motility and indole. In all isolated *E. coli*, were investigated genes associated with virulence factors by Polymerase Chain Reaction (PCR). DNA extraction of *E. coli* strains was performed by boiling and centrifuged at 9676.8xg/2minutes, the supernatant was collected and refrigerated. Specific primers were used to amplify genes encoding virulence factors of adhesin of type 1 pili (fimH), S fimbriae (sfa), fimbriae P (papGI, papGII, papGIII and papC), K99 pilus (k99), afimbrial adhesin (afa), cytotoxic necrotizing factor type 1 (cnf-1), α -hemolysin (hlyA), verotoxin (vt1 and vt2), intimin (eae), uropathogenic specific protein (usp), K capsule (kps), and aerobactin (iucD/aer). To determine the phylogenetic group (A,B1,B2 or D) of *E. coli* strains were used the results of PCR amplification of the chuA and yjaA genes and DNA fragment TSPE4.C2.

Results: *E. coli* was isolated in 35 cultures at Moment 1 and in 24 cultures at Moment 2. Frequencies obtained for fimH, iucD/aer, kps, hlyA, usp, vt1, and vt2 genes were, respectively, 91,4%, 25,7%, 20,0%, 17,1%, 8,6%, 2,9% and 2,9% at Moment 1 and 79,2%, 25,0%, 4,2%, 12,5%, 4,2%, 0% and 4,2% at Moment 2. Sfa, papGI, papGII, papGIII, papC, k99, afa, cnf-1, and eae genes were not detected in any Moment. Distribution of *E. coli* phylogenetic groups in Moment 1 was 54,3% A, 31,4% B1, 0% B2, 14,3% D and in Moment 2 was 58,3% A, 33,3% B1, 8,3% B2, 0% D.

Conclusions: *E. coli* isolated from the uterus until the second week postpartum show virulence factors genes for adhesin of type 1 pili, aerobactin, K capsule, α -hemolysin, uropathogenic specific protein and verotoxin, and belong mainly to phylogenetic groups A and B1. Although

commensal strains, generally, belong to these phylogenetic groups, the presence of several virulence factors may favor the development of uterine infections in a later period depending on the immune status of the cow.

Comments: This work was conducted under the supervision of the ethical use of animals FMVZ-UNESP (protocol number 064/2012).

Reproduction: (Dairy)

P04-004-183

Distal Paravertebral Block for Left Flank Unilateral Ovariectomy in Cattle

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Objectives: Although general anaesthesia is used in cattle, there are some risk with using general anaesthesia. Local or regional anesthesia is safe, effective, and still the most desirable procedure in many situations. Many surgical procedures including unilateral ovariectomy can be performed safely in cattle using a combination of physical restraint, mild sedation, and local or regional nerve blocks. The aim of the study was to obtain the luteal tissue from ovary while defined oestrus cycle time for early pregnancy factors identification with precise molecular biology tools for early embryonic death prophylaxis.

Materials and Methods: The 10 Holstein-Friesian type cows during induced oestrus cycle were enrolled into the study. The elective, surgical procedure under distal paravertebral nerve block was performed in all animals. The distal paravertebral nerve block (between Th13 and L3 vertebra) following pharmacological sedation with xylazine 5-30 mg/100 kg (Xylapan 20mg/ml, Vetoquinol Biowet) combined with physical restraint was performed before left flank ovariectomy. The ovaries were removed to obtain luteal tissue for further detailed examinations. The postoperative antibiotic therapy with procaine benzylpenicillin 1ml/30 kg (Penillin 30%, 300 mg/ml; ScanVet Poland) in combination with postoperative analgesia flunixin 2ml/45kg (Flunimeg 50mg/ml; Scanvet Poland) was proceeding for 3 following days.

Results: No scoliosis and pelvic limb weakness were observed during surgery. Moreover no recumency occurred. Distal paravertebral nerve block provides great quality, unilateral analgesia of the paralumbar fossa for flank laparotomy being alternative to the inverted L block. This block provides uniform desensitisation of the peritoneum than is field infiltration, and also provides relaxation of the abdominal wall. For unilateral ovariectomy, this technique does not risk development of haematomas at the operating site. No general side effects including hypotension or respiratory paralysis were observed after lidocaine administration.

Conclusions: Local anesthetic techniques are usually simple, inexpensive and provide a reversible loss of sensation to a relatively well-defined area of the body including left flank region. Regional or local anaesthesia is safe, effective, and still the most desirable procedure in many situations because standing position in cattle is the best for surgical procedures including unilateral left flank ovariectomy. Properly performer blocks do not desensitize femoral and ischial nerves, thereby preserving pelvic limb function.

Reproduction Technology

P02-002-200

Preselection of high and low ovulatory responders for embryo transfer programs in goats

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Objectives: This study aimed to evaluate the feasibility of establishing a pre-selection test by the use of low dose of eCG to identify does as high or low responders, prior to the use of a multiple ovulation FSH treatment. Concomitantly, the effect of follicular status at the beginning of eCG and FSH-superovulatory treatments and their relationship with the number of corpora lutea obtained was assessed.

Materials and Methods: The study was carried out in a dairy farm at Minas Gerais, Brazil. Twenty Saanen does received 800 IU of eCG at the end of long-term progestagen treatment. Fourteen days later, a second progestagen protocol associated with a multiple-dose FSH treatment (5 IU/kg of FSH, in six decreasing doses between Days 4 to 6 of the protocol) were administered. Transrectal ultrasound was used to assess the ovarian status at the following time: (1) previous to superovulatory treatments to quantify the number of small (2-4 mm), large (>4 mm) and total follicles; (2) onset of estrus behavior to quantify the large follicles; and (3) 7th day of the estrous cycle to quantify the number of corpora lutea (CL). The median of the number of CL per treatment was used as cut-off point for eCG (high ≥ 7 ; low < 7 CL) and to FSH treatments (high ≥ 23 ; low < 23 CL) to characterize the goat donors in high or low ovulatory potential. Other cut-off point was adopted just to compare the number of CL between treatments from goats with a lower number of large follicles (eCG: 5.4; FSH: 4.4) at the onset of the superovulatory treatment. Regression analysis and Pearson correlation coefficient were applied to assess the relationship between the number of CL and follicular status in treatments. The Spearman's rank correlation coefficient and Kappa coefficient were used to evaluate the relationship between high or low ovulatory responder does after each treatment. For all tests, $P < 0.05$ was considered as statistically significant.

Results: Positive correlations were obtained between the number of small follicles at first eCG/FSH dose with the number of CL ($r = -0.47$; $P < 0.05$) as well as the negative correlation between the presence of large follicles at eCG dose and number of CL ($r = -0.44$; $P < 0.05$). Furthermore, positive correlations were also obtained between the number of large follicles at estrus onset with the number of CL (eCG: $r = 0.51$; $P < 0.05$ / FSH: $r = 0.70$; $P < 0.05$). A good multiple correlation between all collected follicular indexes (small follicles, large, total at the beginning of superovulation and large follicles at estrus onset) was obtained for predicting the number of CL ($r^2 = 0.86$; $P < 0.05$). The assessment of the follicular status, prior to superovulatory stimulus, was able to estimate about 50% ($r^2 = 0.48$; $P < 0.05$) in multiovulatory response. A significant relationship ($r^2 = 0.498$; $P < 0.05$) between the number of CL in response to each treatment was obtained from a quartic regression. From the correlation analyzed of preselected goats ($n = 6$), with a lower number of large follicles prior to hormonal stimulation, a linear equation was obtained between treatments ($r = 0.76$; $y = 34.98 - 0.54x$; $P = 0.07$). Overall, 14 does out of 20 still showed high or low response after FSH treatment (70%; 14/20). The Kappa ($k = 0.40$; $P < 0.05$) coefficient did show a moderate concordance regarding to donors to be classified as high or low responders between treatments. However, the Spearman coefficient ($r_s = 0.39$; $P = 0.08$) has just indicated a trend of relationship between them.

Conclusions: It was concluded that the screening protocol with eCG has the potential to identify Saanen goats that will better respond to the

FSH treatment. In addition, it highlighted the importance of an ultrasound assessment prior to the beginning of superovulation treatments with FSH in order to characterize the follicular status and identify potential donors with probable high ovulatory response for MOET programs in goats.

Comments: The results of this study will contribute to the improvement of the current indices obtained in MOET programs in goats. Furthermore, the Buiatrics congress arises as the site for propagation and discussion of the findings with the scientific community.

Reproduction Technology

P02-002-201

Answer Sheep Reproductive Synchronized with Progesterone Utilized Montas Addressed

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Objectives: The aim of this study was to evaluate the response to estrus and treatment costs by comparing two sources of progestogen (progesterone + prostaglandin vs melengestrol acetate (MGA)) in two synchronization protocols

Materials and Methods: 30 sheep were used in the study, which were divided into two batches (L1 and L2). Crosses of Dorper and Katahdin breeds were handled. From 3 to 4 years old, clinically healthy, with an average weight of 36.5 ± 38.2 kg and body condition of 2.5 to 3.5 on a scale of 0 to 5. The treatments were: Lot 1 (Protocol 9 days) was supplied progesterone (P4) on day 1, start of the protocol, with a dose of 0.75 ml IM + prostaglandin (PGF2a) on 9 IM with a dose of 0.75 mg / kg. It was allowed to proceed 24 hours and for the 10th of the protocol, a guard male (with a penis deflected) of the Katahdin breed was introduced, with an average weight of 36.5 ± 37 kg and body condition 3. Lot 2 (Protocol 9 day): melengestrol acetate (MGA) at a dose of 1 mg, and a concentration of 0.44%, orally, following supply MGA was supplied was allowed to elapse 24 hrs, to introduce the male guard (with penis off target) in the Katahdin breed he was introduced. Once the male guard a certain number of days (4 days) was given to the sheep come into estrus in both protocols.

Results: Differences between the two treatments synchronization protocols in the proportion of ewes entered in heat were observed. Lot 1. Of the 15 sheep treated with P4 + PGF2a in this batch, 7 ewes in estrus while the remaining 8 are not presented. The zeal occurred in an average of 66 hours ± 30 hours. Lot 2. Of the 15 sheep treated with MGA (melengestrol acetate) 12 had zeal, while the remaining 3 did not show. The zeal occurred in an average of 45 hours ± 28 hours. Results for percentage of estrus in the operation were significantly different, was obtained for the protocol P4 + PGF2a, a percentage of zeal was 46% and for MGA 80% was obtained. The final treatment interval of estrus in both groups, was a great difference between them, that because the P4 + PGF2a protocol progesterone (P4) is given on day 1 + prostaglandin (PGF2a) on 9 while the AMS was supplied for 9 consecutive days.

Conclusions: Which it concludes the estrus synchronization with melengestrol acetate (MGA) in sheep reproductive induce Positive answers if is APPLY of a high concentration (1 mg / ewe), this benefit producers due to a cost under the (\$ 3.5) Do estrus and Response. While progesterone + prostaglandin has a low reproductive response, if is applies

Concentrations low (0.75 ml P4, prostaglandin 7.5 mg / kg), combined with poor nutrition of (stubble / oats) and Poor body condition 2.5.

Reproduction Technology

P02-002-202

Evaluation of cardiorespiratory parameters in Santa Ines sheep undergoing side access laparoscopic ovum pick-up

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Objectives: The laparoscopic ovum pick-up (LOPU) is widely used in reproductive biotechnology, although this intervention produce physiological changes in the cardiorespiratory system due to Trendelenburg position and CO₂ pneumoperitoneum. Aiming to decrease these problems we development a new surgical approach in the lateral position for follicular aspiration.

Materials and Methods: Twenty-four Santa Ines sheep were randomly divided into two groups with 12 animals: GL - positioned in right lateral position and head down position of 10 degree; GD – supine positioned and an head down inclination of 35 degree. The animals were submitted to LOPU procedure, with 36 hours fasting (hydric and solid), without hormonal treatment, using three portals and CO₂ pneumoperitoneum. The heart rate (HR, beats/minute), respiratory rate (RR, cycle/minute), mean arterial pressure (MAP,mmHg) and pressure at the end of the exhaled CO₂ (EtCO₂) were evaluated in the surgical period.

Results: Lateral and traditionally surgical approaches evaluated in this study were effective for follicular aspiration. Cardiorespiratory parameters were affected by the surgical position being: the Hearth Rate (GL 98,3±3,3 and GD 111±3,6 beats/minute), Medium Arterial Pressure (GL 78±2,3 and GD 87,4±2,6 mmHg) and End Tidal CO₂ pressure (GL 37,3±1,2 and GD 42,5±1 mmHg) were higher in the GL group (p=0,009; p=0,012 and p=0,001 respectively) than in the GD group and the Respiratory rate (GL 40,9±2,1 and GD 36,7±2 mmHg) was similar between groups (p=0,9).

Conclusions: Technique in lateral recumbence for follicular aspiration presents as a viable and effective procedure for oocyte recovery in Santa Ines sheep. However, this access result in considerable cardiorespiratory system alterations when compared to the conventional procedure, making that practical applicability was limited

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Reproduction Technology

P02-002-203

Activation of SIRT1 by resveratrol influences developmental competence of bovine oocytes in relation to maternal age

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Objectives: Maternal age affects the developmental capacity of mammalian oocytes and embryos. Sirtuins are critically involved in ageing associated pathways. SIRT1 belongs to the family of NAD⁺-dependent deacetylases, and it has been shown to regulate several key cellular processes, including transcriptional silencing and ageing. SIRT1 plays also a crucial role in sensing and modulating the cellular redox status. Resveratrol (3,4',5-trihydroxystilbene) is a phytoalexin identified in various plant species which enhances SIRT1 activity by inhibiting phosphodiesterase.

Materials and Methods: The goal of the present study was to examine the effects of resveratrol on SIRT1 gene expression profiles of bovine oocytes and embryos derived from donors of different age. Cumulus-oocyte-complexes of prepubertal (5-6 months old) and adult (2. lactation) cows were collected by Ovum Pick-up. Medium for in vitro maturation and in vitro fertilization was supplemented with 2µM Resveratrol® (Sigma-Aldrich, Buchs, Switzerland). Maturation rates, cleavage and blastocyst formation were evaluated. Comprehensive gene expression assays of germinal vesicle and metaphase II (MII)-stage oocytes and expanded blastocyst were conducted using next-generation sequencing technology.

Results: Relative mRNA abundance levels of SIRT1 were higher in in vitro-matured oocytes derived from cows than in those derived from their younger counterparts (2.8 fold change, p≤0.05). The gene-expression profiles in blastocysts derived from oocytes of the two types of donors showed no significant differences. However, resveratrol improved maturation rates (82.3±4 and 83.6±3 %) and blastocyst formation rates (26.4±3 and 28.7±2 %) in both donor groups compared to a laboratory typical protocol without resveratrol as a control (74.6±5 and 20.8 ±2%, respectively, p≤0.05).

Conclusions: In conclusion, expression profiles for SIRT1 of oocytes and embryos derived from adult donors differ from those of oocytes and embryos derived from prepubertal donors. In addition, activation of SIRT1 in bovine oocytes in relation to maternal age may be a potential indication for determining age-associated events in bovine oocytes.

Reproduction Technology

P02-002-204

Comparison of histology and cytology for diagnosis of bovine subclinical endometritis

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Objectives: Inflammation of the endometrium is a major cause of impaired fertility in the postpartum cow. Particularly, to identify subclinical

endometritis no reliable standardised diagnostic techniques are established. Therefore, it was the aim of this study to compare uterine histology (as the golden standard) and cytology (as a routine diagnostic tool) for diagnosis of bovine subclinical endometritis.

Materials and Methods: Dairy cows from 33 dairy farms were examined by rectal palpation and vaginoscopy at 42 to 50 d postpartum. Inclusion criteria were absence of abnormal vaginal discharge and abnormalities of the uterus (fluctuation) at rectal palpation. Cytobrush samples from the uterus were collected by a modified cytobrush® method. A minimum of 200 cells were counted to determine the percentage of neutrophils (PMN) as indicators of endometritis (thresholds: $\geq 5\%$). Furthermore, biopsy samples from the endometrium were taken using the biopsy forceps according to Kevorikan. Tissue samples were fixed in 4% formaldehyde, embedded in paraplast and stained with hematoxylin-eosin.

Results: Overall, from 277 animals, both types of samples could be obtained. Percent PMN $\geq 5\%$ was 25.9 (n=72). Histologically, endometritis was diagnosed in 105 cases (37.9%). Thirty-three (31.7%) of those samples had a PMN $\geq 5\%$. In 100 samples, a differentiation between purulent (n=91) and non-purulent endometritis was possible (n=9). Percent PMN $\geq 5\%$ was 77.8 vs. 27.5 for purulent vs. non-purulent endometritis (P<0.05). Compared with cows with a negative histological result, cows with non-purulent endometritis did not differ, whereas cows with purulent endometritis had a higher percentage of samples with $\geq 5\%$ PMN (P<0.05).

Conclusions: Our results suggest a relationship between % PMN in uterine cytobrush samples and histologically in diagnosed purulent endometritis. However, using uterine biopsy as the reference method to diagnose endometritis, the calculated sensitivity is just 31.4% and the specificity 77.3% for cytology with a threshold of $\geq 5\%$ PMN. Therefore, with uterine cytology no reliable diagnosis of non-purulent endometritis is possible.

Reproduction Technology

P02-002-205

SEMENRATE: Independent Assessment of Bovine Semen through CASA and Flowcytometry

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Objectives: Conventional parameters routinely examined in bovine semen assessment are concentration, progressive motility and morphology. However, this subjective approach used during quality control at SPCs or during investigations of poor reproductive performance in veterinary practice has been shown to be relatively inaccurate, imprecise and operator dependent (Vincent et al, 2012).

Analysis of semen in use on 50 dairy and beef farms using CASA and flow cytometry in a dedicated breeding laboratory in a veterinary practice aimed to establish variations in the multiple parameters and then evaluate the relationship to semen performance in the field.

Materials and Methods: The development of a new automated independent screening service for frozen and fresh semen, using computer assisted semen analysis (CASA) with flow cytometry (FC) is very new. Initial research was needed to determine the technology and service proposed provides reliable results, as the approach combines 2 emerging technologies (CASA and FC) in a previously unused way.

During this period of the project veterinary surgeons with recognised semen evaluation experience cross checked the results manually. This helps build a multi-parameteric database of results across the EU to enable analysis of the most significant factors to subsequent fertility outcomes on farm.

The 'SemenRate' service will provide farmers with accurate data on semen they purchase to compare and validate semen choice. A funded research study has provided the platform to launch the commercial semen analysis service.

- Stage 1 analysed frozen and freshly collected bull semen from 50 cattle farms against 5 parameters.
- Stage 2 compared analysis parameters to subsequent fertility performance on farm; this is ongoing in collaboration with 7 European countries.
- Stage 3 will extend a commercial service across the UK and EU for freshly collected bull semen, frozen semen collected via a national network of recognized breeding centres associated with XLvets veterinary practices and to semen from other species such as porcine or equine semen.

Results: Three groups of semen (AI frozen, fresh and AI sexed frozen) were assessed using 5 CASA and flow cytometry parameters. For frozen AI semen (n=49), % viable sperm (max=65.22, min=18.80, mean=43.86), % sperm with polarised (active) mitochondria (max=72.50, min=11.25, mean=39.33), % sperm with acrosome intact (max=68.82, min=10.34, mean=36.53), % motile sperm (max=63.80, min=13.10, mean=38.46) and % progressively motile sperm (max=51.60, min=3, mean=25.04).

For fresh semen 5 parameters were assessed (n=48), % viable sperm (max=89.94, min=10.3, mean=50.65), % polarised (active) mitochondria (max=91.56, min=4.3, mean=44.9), % sperm with acrosome intact (max=87.44, min=5.38, mean=41.79), % motile sperm (max=89, min=1.40, mean=47.51) and % progressively motile sperm (max=75.50, min=0, mean=35.65).

For frozen, sexed AI semen 5 parameters were assessed (n=9), % viable sperm (max=66.31, min=17.08, mean=43.57), % polarised (active) mitochondria (max=26.74, min=13.40, mean=19.96), % intact acrosome (max=52.62, min=15.34, mean=37), % motile (max=38, min=9.40, mean=24.88) and % progressively motile (max=22.80, min=3.90, mean=13.15).

Conclusions: An EU collaboration aims to establish correlations between semen quality parameters and fertility outcomes for UK dairy and beef herds providing unique data for British dairy and beef herds. This data should highlight to fellow stakeholders in the industry how imperative optimal semen quality is and highlight the benefits to herd fertility and financial performance.

SemenRate could be used: to screen semen pre ET/OPU-IVF programme; in Bull breeding soundness evaluations for repeatability and objectivity; to screen semen batches to minimise risk of reduced performance & when cold chain is risk high; and to investigate fertility issues.

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Salmonellosis

P04-004-190

A rapid, robust molecular diagnostic test for bovine salmonellosis.

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Objectives: *Salmonella enterica* is a zoonotic pathogen with a broad host range. *Salmonella enterica* serovar Dublin (S. Dublin) is host-adapted to cattle and causes significant morbidity. Infection with S. Dublin can result in long term carriage in cattle, abortion and a high mortality in calves. To enable targeted treatment at the point of decision making, a loop-mediated isothermal amplification (LAMP) assay for the detection of *Salmonellae* is being developed as part of a diagnostic platform. The proposed platform will be rapid, robust, reliable and cost effective in detecting *Salmonellae* in bovine faecal matter for ease of use.

Materials and Methods: To identify highly conserved genes across multiple *Salmonella* serovars, eleven complete salmonella genomes were aligned using a genome alignment software package (Mauve). Using the alignment and relevant literature a number of conserved genes were identified, of which three highly conserved genes were selected for polymerase chain reaction (PCR) primer development, using Primer BLAST. After extensive method optimisation, primer sets have been tested against a panel of eight salmonella serovars, using a no-template assay and *Escherichia coli* as a negative control. Utilising Primer explorer, LAMP primers are being developed for all three target genes using the sequences targeted by the PCR primers. All primers were run through a nucleotide BLAST search, excluding *Salmonella* sp., to test for the likelihood of false positives from the primer set. The matches were assessed by several criteria; whether both primers in a set match the same organism, how exactly the primers matched the genome of the organism and whether that organism is likely to be found in a UK cattle industry setting.

Results: Whilst a full screening of the Mauve alignment was not undertaken, 28 genes conserved across multiple salmonella genomes were found: 7 highly conserved genes were found to be salmonella specific. The 3 genes used for PCR primer development (*hilA*, *orgA*, and *bapA*) were chosen as they are highly conserved across multiple *Salmonella* species, with sparse differences in sequence, and *Salmonella* specific when analysed using BLAST. The *hilA* gene is located within the *Salmonella* Pathogenicity Island 1 (SPI1) and encodes a protein that controls the expression of invasion genes and is required for *Salmonella* to colonise the extracellular surface of the intestinal lumen of the host. The *orgA* gene is also located in SPI1 and encodes a protein involved in the formation of the type III secretion needle structure which aids invasion and secretion. Whilst the function of *bapA* needs further investigation, it is thought to have a role in biofilm formation.

Using the *hilA*, *orgA*, and *bapA* gene sequences 15 sets of PCR primers were generated; 5 per gene. After non-salmonella specific matches were assessed, 6 primer sets in total were chosen for production, 2 per gene. Through PCR it has been established that all 6 primer sets recognised multiple salmonella serovars, demonstrating that multiple *Salmonella* serovars can be identified by targeting these 3 genes.

Conclusions: A rapid, robust and reliable diagnostic platform allows targeted treatment of bovine salmonellosis at the point of decision making. Rapid diagnostics for salmonellosis potentially: reduces economic impacts of the disease; has public health benefits; reduces the spread of infection within herds and environment. Additionally animal welfare is improved as early diagnostics could facilitate the

implementation of targeted treatment so improving cattle health. This is opportune given that multidrug resistance is a worldwide problem. Targeted treatment of disease will promote antibiotic stewardship by reducing blanket treatment in cattle.

Small Ruminants

P01-001-082

The effect of antimicrobial treatment on faecal flora of goat kidsSarah Lais¹Andrea Mueller²Roger Stephan²Cedric Muentener³Ulrich Bleul^{1,*}

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Objectives: The faecal flora of goat kids affected by the floppy kid syndrome consists more of streptococci/staphylococci, enterococci and lactobacilli than the faecal flora of healthy goat kids. Therefore it was suspected that these bacteria might be responsible for the D-lactic hyperemia causing the floppy kid syndrome. Reducing these bacteria might prevent the floppy kid syndrome.

In this study it was tested experimentally, if an oral dose of antibiotic changes the bacterial intestine bacterial microflora in goat kids.

Materials and Methods: Goat kids (n = 29), between 4 and 20 days of age, were randomly divided into three groups and every second day a sample of faeces and blood was taken during an 11-day observation period. Two groups were treated for seven days with two different antibiotics orally. In group T (n = 9) Tylosin, given in a dose of 25 mg per kg body weight twice a day, and in group B (n = 10) Bacitracin, given in a dose of 210 IU per kg weight twice a day, were chosen for antibiotic treatment due to their efficacy mainly against gram positive bacteria. Animals in group C served as controls (n = 10). Every day all kids were examined clinically. The total number of aerobic bacteria and the number of streptococci/staphylococci, of enterococci and of lactobacilli were analyzed in the faecal samples using spread-plate method with selective culture media. A geometric dilution series was used for calculating the number of bacteria per gram faeces based of the number of CFU. D-lactate, blood gas and acid-base parameter were measured in heparinized blood samples. For all groups of organisms, numbers of CFUs were not normally distributed, and therefore groups were compared using Mann-Whitney-U und Kruskal-Wallis tests. A log₁₀(CFU³) transformation was made to test differences over time using ANOVA for repeated measures.

Results: None of the analyzed bacterial groups showed the expected decrease in the amount during the observation period. The numbers of CFU differ neither over time nor between groups. Only on a few single test days significant differences between groups were measured, but in these cases the samples of the control group always showed a lower amount of tested bacteria than the faecal samples of the treatment group. There was no statistical coherence between the amount of bacteria and D-lactate, blood gas and acid-base parameter. None of the goat kids showed clinical signs of floppy kid syndrome. However, on day 0 seven of them showed a concentration of D-lactate > 3 mmol/l in the blood and the concentration decreased significantly until day 8 (p < 0.0001). Simultaneously the pH increased from 7.34 ± 0.071 to 7.42 ± 0.053 (p < 0.05).

Conclusions: The expected decrease of the analyzed bacterial groups due to the antimicrobial treatment was not measurable. The reason for the undetectable effect could not be found. The short duration of treatment, the selected antibiotics or a selection and subsequent growth of resistant bacteria may be possible causes. 24% of the goat kids showed signs of a D-lactate acidosis, which may also have an effect on the faecal flora. Hence, a prophylactic antibiotic treatment as used in this study could not be recommended to prevent floppy kid syndrome in neonatal goat kids.

Small Ruminants

P01-001-083

Effect of a 5-months lameness inspection and gamithromycin treatment on footrot in sheepSimon Rougegré¹Jean-Baptiste Del Marmol¹Benoit Bolkaerts¹Christine Baricalla¹Nathalie Kirschvink^{1,*}

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Objectives: Gamithromycin belongs to the macrolide antibiotics and is characterised by an important volume of distribution and accumulation in skin tissue (Kellermann et al., 2014). It represents an interesting treatment option against ovine footrot that is caused by *Dichelobacter nodosus*. First reports about field studies with short intervention periods describe successful reduction of lameness in affected sheep flocks (Forbes et al., 2014 ; Strobel et al., 2014). The present study describes the long term evolution of lameness and need for treatment in a sheep flock that underwent during 5 consecutive months a weekly lameness inspection.

Materials and Methods: Adult ewes and 4-7 months old ewe lambs of the Ovine Research Center (ORC) of the University of Namur (Belgium) were enrolled in this study (n=427). The sheep flock of ORC was affected by footrot with around 35% of the animals displaying moderate lameness affecting at least one limb. All sheep underwent once per week lameness inspection during which 8-12 animals were observed during 5 minutes. Lame animals received a single gamithromycin injection (6 mg/kg BW SC) and their ear tag number was recorded. The inspection period lasted from July to November 2015. The percentage of treated animals was analysed over time and in function of treatment frequency. 50 animals underwent regular footrot lesion scoring.

Results: 461 treatments were administrated over 7818 individual inspections performed during the observation period. At first week of inspection, 32% of sheep required treatment. During the following weeks, the percentage of treatment requiring sheep ranged between 0% (dry and hot conditions in July) and 15% (rainy conditions in October) with a mean percentage of 4.4% per week. Lameness severity strongly decreased and lesion scores of lame sheep were reduced by more than 50% (passing from a median score of 2 to 1). 33% of the animals did not require treatment during the observation period; 40% were treated once; 16, 8, 2 and 1 % required respectively 2, 3, 4 or 5 treatments.

Conclusions: This study demonstrates that regular lameness inspection and treatment with gamithromycin of lame sheep strongly reduces footrot incidence. However, this lameness-based approach does not allow definite eradication of footrot. Further investigations are necessary to test how eradication of the disease could be achieved, especially by considering sensitive animals requiring more frequent treatments.

Comments: The authors acknowledge Nicolas Noël and Martin Dieu for technical contribution to this study.

Small Ruminants

P01-001-084

Estimation of the influence of risk factors affecting ovine colostrum quality of high producing dairy Lacaune ewesFernando Hernandez¹Laura Torres-Rovira²Laura Elvira³Juan Vicente Gonzalez-Martin⁴Antonio Gonzalez-Bulnes²Susana Astiz^{2,*}

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Objectives: Refractometry using a Brix refractometer has been demonstrated as a means to estimate IgG concentration in bovine maternal colostrum. The refractometer has advantages over other methods being inexpensive, readily available and less fragile, as it has previously confirmed in bovine and ovine colostrum. With the refractometer risk factors affecting colostrum quality can be measured on farm and help to take management decisions quickly.

Objectives: Our objective was to estimate ovine colostrum quality and its variation depending on risk factors declared to influence colostrum quality using an inexpensive Brix refractometer

Materials and Methods: Animals from one single commercial dairy Lacaune farm (4100 ewes; average milk yield per ewe per year of 450L) were included. We tested colostrum from 537 lambings during the years 2011-2013, from ewes with different previous dry period (PDP). A total of 118 were primiparous; 23 had a short previous dry period (0-30 days long); 214 had an adequate dry period (31-60d long); 134 had a long dry period (61-90d long) and 48 had a very long dry period (>90d); parity of the ewes ranged between first and 10th lambing (118 first lambing; 116 second one; 87 3rd one; 84 4th one and 132 ewes had ≥5th lambings); season was also taken into account comparing the lambings in the natural reproductive season (Dec-Aug) versus lambings occurring in the contra-natural lambing season (Sept-Nov). The influence of the age at first lambing was evaluated including the 118 primiparous ewes and separately analyzed. SPSS®19.0 (IBM, NY, USA) was used. The statistical significance of differences was assessed by ANOVA test and relationships between parameters were assessed by Pearson correlation analysis and non-parametric correlation tests.

Results: The average of dry period length was 65.1±41.5d and the age at first lambing 12.7±1.7 mo.

The length of the previous dry period significantly affected the quality on colostrum measured as percentage of solids expressed as percentage by the Brick refractometer. Ewes at first lambing (22.5±5.6%) and those with the longest PDP (20.9±6.3%) showed the maximal qualities (P<0.0001). Ewes with the shortest PDP had the worst quality 16.8±4.2% (P<0.0001). Ewes with an adequate PDP showed 18.7±4.4% and ewes with a long PDP 19.9±5.4%.

Parity significantly affected colostrum quality with the primiparous ewes showing the highest quality (22.5±5.6%; P<0.0001) followed by the ewes with ≥5th lambings (19.9±4.2%). The worst quality was observed by the 2nd lambing ewes (18.8±3.8%).

Year of study was significantly different, with 2011 showing a higher colostrum quality, because we had a bias of the percentage of animals with very long PDP during the year 2011 (21,1 in 2011 vs. 8.9% as average; P= 0.026), bias that was controlled in the analyse.

Lambing season did not affect colostrum quality (20.11±5.0% natural season vs. 19.7±5.7% contra-natural season; P=0.47).

Finally, the age at first lambing showed a quadratic significant relationship with higher colostrum values at the age at first lambing of ≤12mo and ≥15 mo of age (P= 0.012).

Conclusions: These results ratify risk factors such as parity, length of the previous dry period and age at first lambing, affecting ovine colostrum quality of dairy ewes under intensive management conditions. Moreover the Brick refractometer can be used to easily evaluate colostrum quality at ovine dairy farms.

Small Ruminants

P01-001-085

Efficacy of a novel formulation of injectable eprinomectin against natural gastrointestinal nematode (GIN) infections in semiextensive dairy goats

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Objectives: Nematode gastrointestinal infections is an important parasitic problem in goats worldwide. Eprinomectin is the latest avermectin developed to control internal and external parasitosis in cattle. Eprinomectin has a large spectrum, high endectocide efficacy and a low excretion in milk that makes it appropriate for the use in lactating animals. A new eprinomectin injectable formulation has been developed with 0 withdrawal period in milk for dairy cattle. The objective of the present study was to evaluate the efficacy of Eprecis 20mg/ml against natural gastrointestinal nematode infections in goats.

Materials and Methods: From a 400 mixed production goats flock located in Extremadura (Spain) 30 animals were selected. The selected animals had an average milk production of 1.5l/day at the moment of treatment. All animals were naturally infected by gastrointestinal nematodes. Faecal egg counts (FEC) of GIN were performed. The arithmetic average of egg per gram of faeces (epg) excretion of the flock was ≥300. The flock wasn't treated with anthelmintics at least 2 months prior to the beginning of the trial.

Experimental design

Animals were randomly distributed in 3 groups of 10 animals each. G1 (control group/not treated), G2 (animals treated at 0.2mg/kg body weight (bw) subcutaneously), G3 (animals treated at 0.4mg/kg bw subcutaneously).

On D0 animals were treated with the dose determined and on D0, D7, D15 and D30 coprological exams (FEC) were performed (Mc Master, 1970). Coprocultures (Van Wyk, 2004) were also performed to identify the nematodes present by using morphological characteristics as described by Smith, 1981.

Results: All groups had a similar arithmetic average epg on D0 (the day of the treatment): 300 (G1), 345,4 (G2) and 320 (G3). Animals on Group1 (control) showed a continuous eggs excretion after treatment. Arithmetic average epg excretion was 400 on D7, 440 on D15 and 280 on D30.

Animals on Group2 (treated at 0.2mg/kg bw subcutaneously) showed an arithmetic average epg excretion of 0 at D7 and D15 and 54,5 epg at D30.

Animals on Group3 (treated at 0.4mg/kg bw subcutaneously) showed an arithmetic average epg excretion of 0 at D7 and D15 and 20 epg at D30. Both treated groups showed an efficacy of 100% up to D15. On Day 30 both groups had a slight rise on epg with an efficacy <85%.

Conclusions: A novel injectable formulation of eprinomectin (Eprecis 20mg/ml) has been proven to be efficacious on reducing gastrointestinal nematode populations in naturally infested goats flocks at a rate of 0.2 or 0.4 mg/kg bw both applied subcutaneously. The persistency, based on epg counts, was of at least 15 days for gastrointestinal nematode infections. Injectable eprinomectin, can therefore be considered as a new and efficacious tool in the control of nematodes infections in dairy goats.

Small Ruminants

P01-001-086

Transmission of the classical scrapie agent via goat milkTimm Konold¹, Steve A. Hawkins², Hugh A. Simmons¹, Lorenzo Gonzalez³¹Animal Sciences Unit, ²Pathology Department, Animal & Plant Health Agency Weybridge, Addlestone, ³Pathology Department, Animal & Plant Health Agency Lasswade, Penicuik, United Kingdom

Objectives: There is evidence that scrapie, a transmissible spongiform encephalopathy of sheep and goats, can be transmitted from ewe to lamb via milk. This study aimed to determine whether milk collected from goats naturally infected with classical scrapie is also able to transmit the scrapie agent.

Materials and Methods: Milk was collected from eight scrapie-infected goats at clinical or pre-clinical stage and fed to lambs with scrapie-susceptible prion protein genotype (ARQ/ARQ) from one day of age. Lambs were sourced from an established classical scrapie-free flock. The milk from each doe, which included colostrum from two goats, was fed to a pair of lambs (volume ranging from 8 to 87 litres per lamb). Pairs of lambs were kept separate and only mixed if infection was confirmed by immunohistochemical examination of a rectal biopsy. Five ARQ/ARQ sheep were kept in the same building as environmental controls and fed milk replacer. Milk recipient sheep were culled at clinical end-stage and any remaining sheep including controls culled at approximately 1850 days of age. Scrapie diagnosis was made on examination of brain and lymphoid tissues by immunohistochemistry.

Results: Six pairs of goat milk recipient sheep, which had been fed the largest volume of goat milk (38-87 litres per lamb), developed signs of scrapie and were culled at 1122-1552 days of age (mean 1362 days). Scrapie was confirmed by immunohistochemistry. The two pairs of lambs fed approximately 8 litres of milk and culled between 1492 and 1872 days of age and the five control sheep culled from 1858 days of age did not develop signs associated with scrapie, and scrapie was excluded by immunohistochemistry.

Conclusions: Milk from naturally infected goats is infectious and able to transmit the scrapie agent to sheep. Some milk recipient sheep did not show evidence of infection, which may be explained by the lower milk volume consumed, by a lower infectious titre of the milk they received, or by a hypothetical lower susceptibility of sheep to goat scrapie compared to goats.

Small Ruminants

P01-001-087

Field trial to evaluate efficacy of a novel vaccine against biofilm formation by staphylococci, in protecting ewes against staphylococcal mastitisNatalia G. Vasileiou¹, Katerina S. Ioannidi¹, Vasia S. Mavrogianni¹, Dimitris C. Chatzopoulos¹, Dimitris A. Gougoulis¹, Stella N. Kalonaki¹, Teresa Calvo González-Valerio², Roger Guix Vallverdu², Stelios Argyros³, George Fthenakis¹¹Veterinary Faculty, University of Thessaly, Karditsa, Greece,²Laboratorios Hipra S.A., Girona, Spain, ³Laboratorios Hipra S.A., Athens, Greece

Objectives: Objective of the field trial was to evaluate the efficacy of a novel vaccine for protection of ewes against staphylococcal mastitis. The

vaccine induces antibodies against the poly-N-acetyl β -1,6 glucosamine exopolysaccharide, the main component of the extracellular biofilm matrix of staphylococci, and acts in preventing slime production and consequently biofilm formation by these organisms, which are a principal causal agent of ovine mastitis.

Materials and Methods: The trial was performed in an intensively-managed dairy sheep farm in Greece. In total, 150 ewes were enrolled in the study: 76 vaccinated (group V) and 74 unvaccinated controls (group C). The vaccine that was evaluated, is licenced in the European Union (Vimco®; Laboratorios Hipra S.A.) and contains an inactivated slime-producing, biofilm forming *Staphylococcus aureus* strain. Initial vaccination of ewes was performed 6 to 5 weeks before expected lambing date, followed by a repeat vaccination 3 weeks later. Ewes were examined initially once during the first 15 days after lambing and then at monthly intervals up to the end of a 165 day-long lactation period. At each examination, milk samples were collected for bacteriological and cytological examination, which were performed by using established techniques. Blood samples were collected before start of vaccination, as well as 3 and 21 weeks after completion of the full vaccination program from 10 ewes into each group; serum was tested for anti-staphylococcal slime antibodies by means of an ELISA assay. Staphylococcal mastitis was defined as the simultaneous isolation of staphylococci (*S. aureus* or coagulase-negative staphylococci) from a milk sample and presence of either clinical signs (clinical disease) or increased cell content in the sample (subclinical disease). Incidence of staphylococcal mastitis was calculated for the total lactation period. Risk ratios were calculated for that period, as well as for the periods 1-90 and 91-165 days post-lambing.

Results: Fewer cases of staphylococcal mastitis were recorded in group V than in group C ewes (2 versus 3 clinical and 15 versus 23 subclinical, respectively). Incidence risk of mastitis in group V was significantly smaller than in group C: 0.283 versus 0.495, respectively, throughout the trial ($P=0.004$). There was clear evidence that vaccinated ewes were at smaller risk to develop mastitis than controls: throughout the trial, there were 0.00196 cases of mastitis per day at risk of animals into group V and 0.00376 cases for animals into group C ($P=0.017$); respective figures for the first stage of the lactation period (up to 90th day post-lambing) were 0.00209 and 0.00516 ($P=0.004$) and for the second stage of the lactation period (after 90th day post-lambing) were 0.00170 and 0.00048 ($P=0.110$). Further, there was evidence that group V ewes developed mastitis later in the lactation period than the controls: on average the 83rd day versus the 33rd day post-lambing ($P<0.001$). Differences in titres of anti-staphylococcal slime antibodies between ewes in the two groups were not significant before vaccination: median values 5.11 and 3.27 for group V and group C, respectively ($P=0.49$). Differences became significant 3 weeks after completion of vaccination schedule: median values 15.67 and 1.95 for group V and group C, respectively ($P<0.001$); 21 weeks after completion of vaccination schedule differences between groups were significant: median values 5.88 and 0.38 for group V and group C, respectively ($P=0.004$), although in some vaccinated ewes titres had returned to below protective levels.

Conclusions: The results confirmed a protective effect of the vaccine against staphylococcal mastitis. This was stronger up to the 90th of the lactation period. Subsequent waning of anti-staphylococcal slime antibodies corresponded to reduced protection. Protection during the first stage of lactation is important, as at that period there is increased incidence risk of mastitis due to the post-partum reduced immunity often observed in ewes. Milk yield of ewes is highest at that stage, therefore mammary infections have a significant adverse effect both in dairy (reduced milk production) and in meat (suboptimal growth of lambs) production systems.

Comments: The work has been carried out as part of a research grant by Laboratorios Hipra S.A.. The funding organisation did not apply any influence in the experimental design or the presentation of the results of the study.

Small Ruminants

P01-001-088

Efficacy of a novel vaccine against biofilm formation by staphylococci, in protecting ewes against experimentally induced staphylococcal mastitis

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Objectives: Objective of the experiment was to study the efficacy of a novel vaccine for protection of ewes against experimentally induced staphylococcal mastitis and to evaluate potential effects in milk yield of ewes. The vaccine induces antibodies against the poly-N-acetyl β -1,6 glucosamine exopolysaccharide, the main component of the extracellular biofilm matrix of staphylococci, and acts in preventing slime production and consequently biofilm formation by these organisms, which are a principal causal agent of ovine mastitis.

Materials and Methods: In total, 19 ewes were used in the study. They were allocated into group A (n=9; vaccination, intramammary challenge), B (n=3; vaccination, no challenge), C (n=4; no vaccination, intramammary challenge) or D (n=3; no vaccination, no challenge). In ewes into groups A or B, initial vaccination was performed 5 weeks before expected lambing date, followed by a repeat vaccination 3 weeks later; the vaccine that was evaluated, is licenced in the European Union (Vimco[®]; Laboratorios Hipra S.A.) and contains an inactivated slime-producing, biofilm forming *Staphylococcus aureus* strain. In ewes into groups A or C, intramammary challenge was carried out with 10^6 cfu of a slime-producing *Staphylococcus chromogenes*, inoculated directly into the left mammary gland cistern. Clinical examination (results expressed on a 0-5 scale taking into account mammary and systemic signs) and milk sample collection for bacteriological and cytological examination by using established techniques were undertaken daily for 10 days after challenge day and then at weekly intervals for two months after the challenge day. Milk quantity measurements were performed the day before and at weekly intervals after the challenge day. These data referred to milk volume collected from a mammary gland of a ewe over a 4-hour period on each measurement day; comparisons of milk quantities collected were performed (i) between the two glands within the same group, (ii) between groups and (iii) between quantities collected before and after challenge day within the same group.

Results: No adverse reactions were recorded in any ewe after any vaccination. After challenge, clinical signs in vaccinated (group A) were numerically milder than in unvaccinated (group C) ewes: mean score of clinical signs was 0.34 versus 1.50 (P=0.197), respectively. Further, the experimental mastitis was resolved numerically earlier in vaccinated (group A) than in unvaccinated (group C) ewes; duration of disease was 18.7 days versus 31 days (P=0.214), respectively. Before challenge day, average (per ewe) milk quantities collected from the left (LMG) and right (RMG) mammary gland were similar within the same group (P>0.11); there was significant difference among group A and C (120 mL LMG, 128 mL RMG and 152 mL LMG, 152 mL RMG, respectively; P=0.045), but not among other groups (P>0.12). After challenge day, average (per ewe) milk quantities collected from LMG or RMG were similar within groups A, B, D groups (P>0.10), but not within group C (76 mL LMG versus 91 mL RMG; P=0.04). Furthermore, the significant differences observed among group A and C before challenge day had not been apparent after that (P=0.49), as decrease on milk quantities collected was greater in group C ewes (average milk quantity per ewe on the challenged mammary gland: 66.5 mL) compared to group A ewes

(average milk quantity per ewe on the challenged mammary gland: 40.0 mL) (P=0.035).

Conclusions: There is no documentation available regarding efficacy of this licenced vaccine against experimental intramammary challenge of ewes with staphylococci directly into the mammary parenchyma and effects on milk yield of vaccinated animals. The results confirmed the protective effect of the vaccine, as evidenced by the milder clinical signs, the earlier resolve of the disease and the smaller decrease in milk yield compared to results of challenge of unvaccinated ewes.

Comments: The work has been carried out as part of a research grant by Laboratorios Hipra S.A., The funding organisation did not apply any influence in the experimental design or the presentation of the results of the study.

Small Ruminants

P01-001-089

Prevalence and ultrasonographic characteristics of lung and pleural pathologies that may affect the identification of ovine pulmonary adenocarcinoma.

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Objectives: The objective of this study was to determine the prevalence and sonographic appearance of lung and pleural pathologies that may complicate the ultrasonographic diagnosis of naturally occurring ovine pulmonary adenocarcinoma (OPA) in adult sheep in the United Kingdom.

Materials and Methods: Adult sheep in four flocks (comprising 350, 500, 700 and 850 breeding ewes, respectively) in the United Kingdom were screened for ovine pulmonary adenocarcinoma (OPA) during autumn 2015 using a 6.5 MHz microconvex probe connected to a real-time, B-mode ultrasound machine. Trans-thoracic ultrasonographic examination of both sides of the chest was undertaken by the same operator (PS) at a rate of 50-80 sheep per hour. All sheep with OPA were culled from the flocks and necropsied. A retrospective analysis was also undertaken of 62 adult sheep with lung and/or pleural pathology submitted over a three years' period (2013-15) to the University of Edinburgh Veterinary School's Farm Animal Hospital (FAH).

Results: Two sheep with extensive unilateral fibrinous pleurisy were identified in the 700 ewe flock during the OPA screen; neither sheep showed signs of illness. Three sheep with fibrinous pleurisy were diagnosed ultrasonographically in 62 adult sheep presented to the FAH. Four sheep were treated with procaine penicillin daily for four weeks; three sheep responded well. A sharply-demarcated hypochoic area in the ventral margin of the lung field in three of these five sheep was considered to be consolidation rather than OPA because these lesions could no longer be imaged three (1 sheep) and six weeks (2 sheep) later. Six of 55 sheep with OPA in the flock screening study had extensive secondary abscessation of the OPA tumour affecting one side of the chest. Extensive secondary abscessation of OPA lesions identified during ultrasound examination of four of 52 sheep in the FAH study was confirmed at necropsy. Typically, in these 10 cases the dorsal margin of the lung lesions appeared as sharply-demarcated hypochoic areas with abscesses present as 2-3 cm diameter poorly defined anechoic areas containing multiple hyperechoic dots more ventrally. A diagnosis of OPA in all the secondary abscessation cases was made possible by finding smaller OPA lesions without secondary bacteria infection in the contralateral lung.

Conclusions: Lung consolidation imaged in three of six sheep with extensive fibrinous exudate could not be differentiated sonographically from OPA pathology therefore such sheep should be isolated, treated with antibiotics, then re-scanned 6 weeks later. Where ultrasound examination of the more ventral lung field identified multiple abscesses within consolidated lung tissue in 10 sheep, examination of the dorsal margin of pathology imaged the typical sharply demarcated pattern of OPA as well as finding typical OPA lesions in the contra-lateral lung.

Small Ruminants

P01-001-090

On-farm ultrasonographic examination of the lungs of sheep in a pilot programme to control ovine pulmonary adenocarcinoma (OPA) in affected flocks

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Objectives: The objectives of this pilot study were to evaluate the sensitivity and specificity of high throughput trans-thoracic ultrasound scanning of the chest of sheep (50-80 sheep scanned per hour per veterinary surgeon) to detect OPA lesions in commercial sheep flocks. A benefit:cost analysis was undertaken after the first screening in four flocks to provide financial data for veterinary practitioners and farmers.

Materials and Methods: An initial prospective study had examined the authors' accuracy of trans-thoracic ultrasound examination using a 6.5 MHz sector ultrasound machine widely available in veterinary practice in the United Kingdom (DP50; BCF Technology) to diagnose OPA lesions >3 cm diameter in contact with the visceral pleura (Cousens and Scott, Vet. Record 2015;177:366). Handling, preparation and examination time was restricted to 5 minutes per sheep. One hundred sheep were examined; all 41 cases identified with suspect OPA lesions during trans-thoracic ultrasound examination had the diagnosis confirmed at necropsy, whilst sheep without ultrasonographic changes characteristic of OPA had no gross lesions of OPA at necropsy.

Subsequently, 50-80 sheep were scanned per hour by one veterinary surgeon or 50-100 sheep scanned per hour by two veterinary surgeons in four large commercial flocks (350-910 breeding sheep). Sheep with well-defined hypoechoic lesions measuring >1 cm with the echogenic appearance of previously confirmed OPA cases were deemed positive and culled from the flocks to expedite removal of as many potential OPA cases as possible. Necropsies of suspected OPA positive and negative cases were undertaken where possible. Local market prices for cull ewes at the time of examination were used in the benefit:cost calculation taking into sheep body condition score and liveweight. No allowance was made for projected reduced losses following lowered transmission of JSRV virus after removal of OPA-affected sheep.

Results: In flock A, one of 15 suspect OPA positive sheep was negative at necropsy; no lesions were found in the 12 predicted negative sheep. In flock B, one of 15 suspect OPA positive sheep was negative at necropsy; one of four predicted negative sheep had a 1 cm OPA lesion. No OPA cases were detected in flock C. Only 2 of 12 sheep from flock D were necropsied confirming OPA in both cases. Only 4 of the 21 OPA sheep in flock A and 3 of 26 OPA sheep in Flock B were picked out by the farm staff.

Estimated benefit:cost Flock A -£105, Flock B +£542, Flock C -£350 Flock D -£196.

Conclusions: The sensitivity and specificity of diagnosis was lower when operating a high throughput ultrasound scanning system where lesions >1 cm were considered as OPA positive. In negative and low prevalence flocks the cost of scanning was approximately 25 pence to £1 per ewe whereas in high prevalence flocks there would be an immediate financial benefit because sheep with early lesions could be sold for slaughter and furthermore did not incur disposal charges.

Small Ruminants

P01-001-091

Modelized evolution of Schmallenberg virus seroprevalence in a sheep flock

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Objectives: The research sheep flock of the University of Namur was infected by Schmallenberg virus (SBV) in autumn 2011 leading to 98% (417/422) SBV seroprevalence and underwent SBV re-emergence between July and October 2012. Since then, no serological evidence of SBV novel episode has been detected increasing yearly the number of seronegative animals and probably promoting future viral re-occurrence. The objective of this study was to modelize the evolution of SBV seroprevalence in this flock by considering the duration of colostral protection in lambs and active immunity in adult sheep.

Materials and Methods: Two groups of animals were used. The group A (n=30) was composed by lambs born in 2013 from SBV infected ewes and the group B (n=50) was composed by adult sheep that encountered SBV infection in 2011 or in 2012 during viral re-emergence. Blood was collected in those two groups at different time points. In order to evaluate the decay of colostral antibodies against SBV, group A animals were collected at birth, 48h after first colostrum intake and then bimonthly until 150 days after birth (DAB). To assess the persistence of active immunity against SBV, group B animals were collected quarterly from early 2013 until late 2015. Anti-SBV antibody titers were evaluated in animals' serum by virus neutralization tests (VNT) and expressed as the dilution neutralizing 50% of the challenge virus (ED50). Animals were considered seropositive if the log² ED50 was > 3.49.

Results: In lambs, the median (min – max) length of time between birth and the first detection of VNT negative result was 122 days (74 – 144). In adult sheep having encountered SBV infection, all the 50 animals remained seropositive from early 2013 until late 2015. Median (min-max) log² ED50 anti-SBV antibody titers in early 2013 equaled 8.97 (6.48 – 10.96) and were significantly higher than titers obtained at the following sampling times that equaled 8.47 (6.47 – 8.47) in early 2014, 8.47 (6.47 – 8.47) in early 2015 and 8.47 (6.47 – 8.47) in late 2015 respectively (p<0.05). Based on these results, lambs born from SBV seropositive ewes infected by the virus in 2011 or in 2012 were considered to benefit from colostral protection until 4 months of age. Adult animals that encountered SBV infection in 2011 or in 2012 were considered seropositive from their time of infection to date. Animals born after the period of SBV re-emergence (after October 2012) were considered seronegative as their lambs were deprived of colostral protection against SBV. By considering these data, lambing periods (4/year) and the renewal in this research sheep flock, the evolution of SBV seroprevalence was modelized. In early 2013, the estimated within-flock SBV seroprevalence equaled 91% (669/735) while it equaled 76%

(509/669) and 50% (235/474) in early 2014 and late 2014 respectively. In autumn 2015, the SBV seroprevalence dropped to 37% (152/410).

Conclusions: In conclusion, based on calculated duration of colostral protection in lambs and evaluation of long-term immunity in adult sheep, the evolution of SBV seroprevalence was modeled at a herd scale. It appears that the absence of novel SBV circulation since 2012 associated with flock renewal has clearly increased the number of seronegative animals as soon as colostral protection against SBV disappears. This results in supposed high flock susceptibility to SBV infection. The accuracy of this model needs to be confirmed by VNT among the entire sheep flock.

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Small Ruminants

P01-001-092

Association of milking parlours and milking procedures with subclinical mastitis in dairy sheep

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Objectives: The standard operating procedures of machine milking are usually underestimated or ignored by the majority of dairy sheep farmers. As a result, udder health problems and large variation in milk quality dominate the routine of dairy flocks. The objective here was to assess the role of milking parlours on udder health and particularly the prevalence of subclinical mastitis in dairy sheep.

Materials and Methods: A random number of twenty-eight dairy sheep farms, located in Northern Greece, were used. Each farm was visited by a team of two veterinarians that assessed the structure and function of the milking parlour. A designated recording form was used to assess the following: i) type and structure of the milking parlour, including dimensions and holding area, numbers of stalls and milking units, diameters of pipelines, number of milkings per day, frequency of teat cup liners replacement and general maintenance. Moreover, a specific mobile device (Exendis Milking System Analyser PTV) was used to measure the vacuum level in the clusters (in order to compare it with the vacuum of the pump), the pulsation rate and the duration of pulsation phases, in three milking units of each line in every milking parlour. A random sample of milking ewes from each flock, representing about 20% of the total population in the flock, was selected for milk sampling in order to perform California Mastitis Test (CMT; scores 0-4) as an indicator of subclinical mastitis. If the percentage of ewes with milk assigned CMT score 3 and 4 was over 20%, then the flock was considered as positive for subclinical mastitis. Data were analysed using univariate analysis of variance with SPSS 22 statistical software. Vacuum level, difference of vacuum level between clusters and vacuum pump, pulsation rate, pulsation ratio and cluster removal after vacuum cessation were transformed into categorical variables and used as fixed effects.

Results: In 71% of the flocks, the percentage of animals with CMT 3 and 4 was over 20%. The average percentage of ewes with CMT 3 and 4 was 7% and 22%, respectively. The holding area was small compared to the number of milking ewes in the majority of the flocks. Also, the length of milking stalls was < 90cm in 36% of the flocks and the width was < 35cm

in 25% of the flocks. Additionally, in 14% of farms, the diameter of pipes in the milk line was smaller than normal, which resulted in a decrease in vacuum level and acts as a predisposing factor of subclinical mastitis. At only 57% of the flocks the milking machine maintenance was done by a specialist. Furthermore, only one farmer replaced the liners after 2500 milkings; all others were well above this number. In 79% of farms the milking procedure was considered inappropriate. The effect of ewes per milking unit was statistically significant, with the flocks that had more than 11 ewes per unit showing a higher percentage of subclinical mastitis (24.5% to 31.4%, P=0.012). The vacuum level measured in clusters differed significantly from that in the vacuum pump (≥ 2 kPa) at two or more measurements in 50% of flocks and at one measurement in 21% of flocks. In case such difference existed at two or more measurements, subclinical mastitis was observed in a significantly higher percentage (33.5%) than in the other cases (24.7%, P=0.047).

Conclusions: Errors in milking process and lack of proper maintenance of the milking parlour increased the prevalence of subclinical mastitis in dairy sheep. Farmers should have the milking parlours serviced by specialists in order to limit any issues that have negative consequences on animal health and welfare.

Small Ruminants

P01-001-093

Usefulness of clinical observations and blood chemistry values for predicting clinical outcomes when treating dairy goats with pregnancy toxemia

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Objectives: The objective of this study was to establish a prognostic algorithm for dairy goats with pregnancy toxemia (PT) based on the blood values for pH, beta-hydroxybutyric acid (BHBA), glucose, ions (K⁺, Na⁺, Cl⁻), blood gases and clinical signs.

Materials and Methods: This study was done on a 1,800-head dairy goat commercial farm. The goats were of two breeds: Alpine and Saanen. The present study involved a total of 32 goats. All the goats included in this study were dry goats.

Any pregnant doe in the dry doe group that refused to eat when fresh feed (concentrate) was offered (4 times during a 24 hour period) was considered to be a PT suspect and her blood BHBA value was determined using an electronic on-farm test (Precision Xceed®, Abbott, UK). If this value was found to be 3 mmol/L, or greater, she was considered a PT doe and handled accordingly.

At the time that each PT doe was enrolled in this study, a physical examination was performed, clinical signs (of disease) and vital signs (rectal temperature, heart rate, respiratory rate and ruminal motility) were determined and recorded. In addition, a blood sample was obtained from the jugular vein with a 20-gauge needle using a 1 mL new syringe and the following parameters ions (Na⁺, K⁺, Cl⁻ and HCO₃⁻), glucose, pH, BE (Base Excess), pCO₂ and BUN were measured on the farm, using a portable analyzer (i-Stat®, Sensor Devices Incorporated, Waukesha, WI; USA):

Consent was obtained from the owner of the animals prior to their enrollment in the study. All the experimental procedures (handling, blood sampling, and caesarean when applicable) were performed so as to

minimize the stress and discomfort experienced by the animals.

Results: Polypnea (88%), swollen limbs (78%), anorexia (69%), recumbency (66%), neurological signs such as "star gazing" or opisthotonus (9%) and drooped ears (9%) were the most observed clinical manifestations in the 32 PT goats. In the present study, the case fatality rate was 75% (24/32).

Nineteen out of 21 recumbent goats died. Sixteen out of 17 goats with anorexia died. All the goats that showed neurologic signs (3 out of 3) and drooped ears (3 out of 3) died. Mean BHBA and glucose values in the goats that died were not significantly different from those in goats that survived (6.5 vs 6.0 mmol/L and 2.2 vs 2.0 mmol/L, respectively). The blood values for pH (7.13 vs 7.30), pCO₂ (26 vs 33 mmHg), HCO₃⁻ (8 vs 21 mmol/L), BE (-20 vs -5 mmol/L) and K⁺ (2.7 vs 3.7 mmol/L) were significantly lower in the goats that died.

Conclusions: The results showed that anorexia, recumbency, neurological signs and drooped ears can be used as prognostic indexes in goats with PT. Among the blood parameters to be considered, K⁺ and those indicative of a metabolic acidosis can also be used as prognostic indexes. The other blood parameters measured in this study (Na⁺, Cl⁻, glucose, BUN and BHBA) do not have prognostic value in goats with PT.

Small Ruminants

P01-001-094

Case study on the usage of Gamithromycin for the treatment and prevention of ovine caseous lymphadenitis (*Corynebacterium pseudotuberculosis*).

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Objectives: Caseous lymphadenitis (CLA) is an infectious disease caused by *Corynebacterium pseudotuberculosis*. The objectives of this case study were to assess if a single injection of Gamithromycin (GAM) subcutaneously could improve the recovery process and case management associated with the more common and less severe CLA cases seen in a Moroccan 1000+ sheep feed-lot where routinely 40 to 60% of the animals are affected with CLA and, if so, to assess the potential for GAM to be used to reduce the prevalence of the disease in the flock.

Materials and Methods: Individual animals affected with CLA, in Morocco, are treated as required with either antibiotics and/or surgical draining and flushing of the abscesses. This approach is intensive and many treated cases suffer protracted periods of recovery. Further, the wounds can also be a source for on-going infection of the flock. In 2013, thirty sheep of different ages (average weight of 60 kg) were included in the study on the basis of having a detectable and non-ruptured small to medium sized abscess on D0 and on not having received any prior antibiotics to treat the condition. All sheep were injected subcutaneously on day 0 with Gamithromycin (Zactran®) using a consistent dose of 4 mL (10 mg/kg). The pharmacokinetics of Gamithromycin make it potentially ideal for managing CLA cases, with a tendency to concentrate for persistent periods in inflamed tissues. Each animal was then closely monitored over a 30-day period post-treatment. The information collected for each case at each time point were: Lymph node affected, Size of swelling at D0 (Small, Medium, Large, or Very Large), Degree of change in swelling on days 5, 15 and 30 (0 = no improvement to 5 = resolved) and detection of abscess rupture. This type of monitoring allowed for

at least a semi-quantitative determination of the level of improvement for each case and also for an assessment of the speed of response. Towards the end of 2014, the entire flock was treated at the same time with a single dose (6 mg/kg) of GAM.

Results: All 30 cases had small to medium-sized abscesses in either the parotid (12) or retropharyngeal (18) regions. Six sheep also had large to very large abscesses in either the breast/chest region (1), the groin/inner thigh region (2) or the gluteal/perineal region (3). All assessed abscesses showed significant reduction in size by day 30 (with no rupture) and were noted as improving in size from the first post-treatment visit (day 5). All swellings were still detectable on day 30 but the vast majority were graded as 4 (the last grade prior to complete resolution). Whilst culturing abscess material was not part of the study, *C.pseudotuberculosis* has been routinely isolated from affected sheep on this farm, including one of the included cases that was sampled during this study. In 2015, following the whole flock treatment in 2014, there were 20 abscess cases identified (2-5%).

Conclusions: On this farm, all small to medium-sized lesions showed significant improvement without rupturing or requiring surgical intervention, virtually resolving by the end of the study period. The response to the whole flock treatment was also impressive with a dramatic reduction in flock incidence being seen in the following year. The results seen were positive and support further studies to both confirm these findings and to better define both the type of cases suitable (e.g. site and severity) and the dosing regimen(s) required. The recommended label dose for cattle is 6 mg/kg.

Comments: Zactran is a registered trademark of Merial Inc.

Small Ruminants

P01-001-095

Ovine Herpesvirus 2 In Goats In Austria: Epidemiological And Case Study

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Objectives: Ovine herpesvirus 2 (OvHV-2) belongs to the genus Macavirus, in the subfamily Gammaherpesvirinae. OvHV-2 is worldwide endemic, mainly in sheep but also in goats. It is well adapted to these species and does not induce any clinical signs. In clinically susceptible hosts, such as cattle, bison, deer and buffalo an OvHV-2 infection causes Malignant catarrhal fever (MCF), a disease that usually has a fatal outcome (Russell et al., 2009).

Materials and Methods:

Epidemiological study

The study was carried out among 100 goats, being patients of the University Clinic for Ruminants of the University of Veterinary Medicine Vienna. Blood samples collected from the animals were investigated for OvHV-2 specific DNA using PCR.

Case study

A female dwarf goat with a five week history of alopecia and crusting dermatitis admitted to the Veterinary University Clinic for Ruminants. Moreover, 12 contact sheep and two contact goats. All animals were clinically examined and blood samples as well as conjunctival and nasal

swabs were collected and investigated for OvHV-2 specific DNA using PCR.

Results:

Epidemiological study

OvHV-2 specific DNA was detected in 7 (7%) blood samples. The 7 animals (6 female, one male) were between 1 and 14 years old. Clinical signs typically for MCF were not seen in these animals.

Case study

The goat described was the only affected animal in a flock harbouring 12 sheep and 2 goats. At admission clinical examination revealed that the goat was mildly depressed and dehydrated with an increased body temperature and heart rate. Conjunctivitis, photophobia, keratitis and anterior uveitis were diagnosed in both eyes. The hair coat was generally dull and rough with hairless areas. Patches of the skin were hard, dry, hyperkeratotic, painful and locally fissures and ulcerations were seen. After 12 days of hospitalisation the goat developed nervous signs and was euthanized. Pathology, histopathology and virological investigations confirmed the suspected diagnosis OvHV-2 infection.

The remaining animals of the flock were clinically healthy. In blood samples of eight sheep and one contact goat OvHV-2 specific nucleic acids were found. Moreover investigations of conjunctival /nasal swabs showed in one sheep a positive result. Sequence analysis performed on OvHV-2 positive samples showed a 100%identity between those from the animals of the flock and that one of the clinically affected goat (Bagrinovschi et al., 2014).

Conclusions: Only a few epidemiological studies according the dissemination of OvHV-2 in goats are described worldwide. Among the animals investigated in the study presented here the infection rate with OvHV-2 was 7%. To determine the importance of goats in the spread of OvHV-2 among ruminants in Austria further studies are needed. However, clinical signs in goats affected with OvHV-2 reported here are rarely described in the literature. This was the first case of an OvHV-2 infection in a goat in Austria characterised by clinical signs.

Small Ruminants

P01-001-096

Determination of risk factors associated with postparturient udder edema in dairy goats

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Objectives: Udder edema is one of the major problems in dairy goats imposing colossal economic loses to the farming community by affecting milk production particularly in heavy milker goats. The objective of the present study was to determine the risk factors associated with postparturient udder edema in dairy goats at different farms in lahore, Pakiatn.

Materials and Methods: A prospective study was carried out in which the data was evaluated to determine risk factors associated with udder edema in dairy goats. Three hundred and fifty (n=350) dairy goats either pregnant, near to parturition or recently parturited were considered

for this study. Udder edema was diagnosed on the basis of visible and palpable changes in the udder tissue. Goats with enlargement of udder with evident skin distension, fluid accumulation and retention of finger imprint were considered as case of udder edema Time to return to normal of fingerprint of the skin of the udder for more than 3 sec confirmed the diagnosis. Variables considered as potential risk factors were breed, age at kidding, kidding month, doe weight, height at the level of shoulders, body condition score at kidding, kidding difficulty, retention of placenta, number of gestation, previous history of udder edema, number of off-springs, gender of off-springs, milk yield, housing system, feeding system and type of feeding. Each of the factors included were evaluated by Chi square test. Adjusted odds ratios (OR) and 95% confidence intervals (95% CI) was reported.

Results: Goats fed on more salt than recommended during pregnancy were 18.08 (OR) times more likely to develop udder edema than goats not fed to salt. Goats giving birth to male kid are at greater risk. Recently parturited goats (1-2 day) were 1.18 times more susceptible to udder edema compared to goats 3-5 days after kidding. On the other hand, goats that were pregnant >4.5 m (near to parturition) were 0.43 times more prone to udder edema than goats of <4 m pregnancy. Younger goats were 1.263 times more likely to develop udder edema than older ones (P>0.05). The goats in first Parity were 1.26 times more likely to develop udder edema than goats in 2nd parity and so on. Occurrence of udder edema was 1.54 times higher in goats kidding during the month of January compared to November-December and February-March. Goats having kidding difficulty (dystokia) were 3.60 times more likely to contract udder edema than goats having normal kidding (P<0.05). Goats with retained fetal membranes were 2.27 times more prone to udder edema than without RFM. Goats having previous history of udder edema were 3.26 times more susceptible to develop udder edema than goats which did not develop udder edema previously (p<0.05). It was found that as the number of the off-spring increased chances of udder edema were also increased. Results showed that if there were twins then chances of udder edema are increased by 0.65 times. It is also statistically significant when compared a single kid with twins (P<0.05) but it is statistically non-significant when twins and triplets were compared (P>0.05).

Conclusions: It was concluded that several risk factors like high salt concentration in feed, age of the goats, parity, retained fetal membrans, number of kids at birth and sex of the kid are significantly associated with udder edema in dairy goats.

Comments: This paper represents novel information about risk factors of udder edema in dairy goats and is first ever study in Pakiatan.

Small Ruminants

P01-001-097

Environmental and management influences on Haemonchus contortus prevalence on North Carolina goat farms

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Objectives: The objective of this study was to identify factors contributing to H. contortus presence on North Carolina goat farms, and identify parasite reduction strategies that are effective in controlling H. contortus infection.

Materials and Methods: Fecal samples were collected from 560 adult goats from 51 different farms. Goat farms were identified from hospital, producer group, and North Carolina Department of Agriculture records. Farms were solicited by postal mailings, and participating farms were visited to collect fecal samples and administer a survey designed to assess potential risk factors. A McMaster's exam was performed on each fecal sample to determine fecal egg count (FEC). A second survey was administered to elicit more quantifiable data after analysis of initial survey.

Results: Univariate analysis revealed farm-type, herd size, anthelmintic use, pasture type, presence of a natural water source, raised feeding trough use, and owner perception of difficulty controlling parasitism to be significantly associated with average farm FEC. Age, grazing environment, pasture grass height, herd introductions, breed, goat use, kidding, lactation, diet, and coccidia prevalence were significant in predicting individual goat FEC. Multivariate analysis revealed breed, goat use, anthelmintic dosing strategy, daily dietary protein intake, Coccidia presence, age, kidding and lactation, and dew exposure to be significant in predicting FEC.

Conclusions: Results identified young age, recent kidding, and lactation as risk factors predisposing goats to *H. contortus* infection, while being a companion animal appeared to have a protective effect. Farms who never use chemical anthelmintics have the lowest FECs suggesting that alternative parasite control measures may be effective, and that preventative measures including grazing wooded areas may reduce the need for anthelmintics, and subsequently slow the development of anthelmintic resistance.

Small Ruminants

P01-001-098

Survey on "Abscess syndrome" in small ruminants in Emilia Romagna Region (Italy)

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Objectives: The presence of abscesses is a widespread problem in sheep and goat, and *Staphylococcus aureus* subsp. *anaerobius* (SAAN), agent and *Corynebacterium pseudotuberculosis* (CPS), are the recognized agents of "Abscess disease", also known as Morel's disease (MD), and of caseous lymphadenitis (CLA) respectively. The objectives of this study were to evaluate clinical appearance and spread of these diseases in farms of Emilia Romagna Region and to characterize the aetiology of the clinical cases observed.

Materials and Methods: In this seven-month study, 10 farms who referred abscess presence were visited, including eight dairy goat farms which held Chamois colored goats and two sheep farms which held Sarda (milk) and Suffolk (meat) sheeps, with the average age of 7 years. Sixteen animals (14 goats and 2 sheeps) with abscessual lesions were examined and pus samples were collected by 5-10 ml syringes with 18-gauge needle, then sent for bacteriological investigations to Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Bologna department. Every sample was concurrently subjected to a bacteriological examination by direct smear on Blood Agar plates and enriched in a liquid medium Brain Heart Infusion (BHI); for every sample a Blood Agar plate was incubated at 37°C in an aerobic atmosphere and

one in an anaerobic atmosphere using a commercial kit (GENbag anaer, Biomerieux). Blood Agar plates were observed daily for a maximum period of 72 hours, then developed grown colonies were identified by fenotypic evaluations (colonial appearance, hemolysis, gram staining, urease/catalase/coagulase and other biochemical tests).

Results: Clinical examination revealed the presence of round to oval lesions, singles and rarely multiple, with smooth and uniform surface, not warm nor painful, larger ones located usually in the neck and sternal regions. The abscessual lesions were mostly located in prescapular and subauricular regions as well in head and precrural region, neck and sternal region, ischiatic region and their size ranged between 1,5-10 cm. Animals overall body condition score was rated 2/5, probably due to CAEV presence in farms. The bacteriological examination revealed the presence of the typical agents causing abscesses like SAAN and CPS but, surprisingly, in 48% of collected samples, other bacteria was isolated like *Trueperella pyogenes*, *Staphylococcus aureus*, *Kocuria kristinae*, *Staphylococcus caprae*, *Streptococcus thoralthensis*, *Prevotella*, *Peptoniphilus asaccharolyticus*. Only in 5 cases we found a mixed infection. Some of these last species didn't previously described as cause of abscess disease in small ruminants in Italy. Furthermore it is worthwhile to note that these "non conventional" bacteria are commonly isolated in humans.

Conclusions: The results show heterogeneity of bacteria that can cause abscesses and this must be considered in the preparation of field vaccines. The key points are biosecurity measures and farm management: care of animals with abscesses to prevent spontaneous breakage and contamination of environment and livestock facilities; strong plans against flies to limit the spread of bacteria. Since we found a high prevalence of abscess caused by non conventional species of bacteria, we propose the labeling of "Abscess Syndrome" to distinguish them from the classical form know as Abscess disease and CLA.

Small Ruminants

P01-001-099

Malignant Catarrhal Fever In Farmed Cervids In Texas

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Objectives: Malignant catarrhal fever (MCF) is a member of the gammaherpesvirus group that contains several pathogenic viruses that affect a number of susceptible ruminant species. Malignant catarrhal fever has been reported in at least 14 species cervids. Cases from the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) involving MCF in cervids were reviewed to determine common clinical signs, gross and microscopic lesions, and association with known MCF reservoir ruminant species in order to assist with management of cervids and other exotic ruminants.

Materials and Methods: Cases involving the diagnosis of MCF in cervid species was reviewed to determine common clinical signs, gross and microscopic lesions, and real time PCR results. Gross lesions were recorded for necropsies performed at TVMDL. Tissues were collected from all major organs and half were fixed in 10% buffered formalin and half were saved as fresh for further testing if needed. Fixed samples were processed following standard procedures for histopathology. Samples for realtime polymerase chain reaction (qPCR) testing for MCF were prepared following accepted molecular techniques. Clinical signs were recorded as submitted by the referring veterinarian. Case which were

qPCR positive for MCF were analyzed to establish if common clinical signs, gross and microscopic lesions, and exposure to MCF reservoir species existed.

Results: During the two-year period of 2014-2015 MCF was diagnosed at TVMDL in 5 white-tailed deer and 2 axis deer from game ranches in Texas. Clinical signs ranged from listlessness, anorexia and progressive decline over three week period leading to neurological disease and death. Other affected deer showed signs of acute neurological disease. Other deer showed a more protracted disease which lasted several days before terminating in death. Gross lesions included organ enlargement, epicardial petechia, lymphadenopathy and serosal hemorrhages. The microscopic lesions included peri-arteritis and arteritis, necrotizing endarteritis, lymphoid hyperplasia and lymphocytic/mononuclear inflammation of various organs. Two MCF viruses were confirmed by qPCR; these viruses were ovine herpesvirus-2 and MCFV-Ibex.

Conclusions: Deer affected with malignant catarrhal fever can exhibit various signs which can be acute in onset. The clinical signs and gross lesions can be confused with other disease conditions affecting cervids. Definitive diagnosis of MCF must be by histopathology and/or qPCR. Susceptible cervid species are at risk of contracting MCF when pastured with or adjacent to ruminant species that are known reservoirs for the MCF virus.

Small Ruminants

P01-001-100

Strategic control of sheep gastrointestinal nematodes using *Duddingtonia flagrans* and Levamisole Hydrochloride 5%

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Objectives: The objective was to evaluate the action of *D. flagrans* pellets associated with strategic anthelmintic treatment with Levamisole Hydrochloride 5% in the control of sheep gastrointestinal nematodiosis in the Brazilian semiarid.

Materials and Methods: Were used 18 Dorper sheep, females, aged between 24-36 months and a mean weight of 50 kg. It was created three groups consisting of six sheep. In group 1, each animal received 3g of the pellets (0.6g of *D. flagrans* mycelium) for each 10 kg of body weight, twice a week for six months and a deworming with Levamisole Hydrochloride 5% each EPG \geq 1500 (Fungi + Chemical). In group 2, each animal received a dosage of levamisole hydrochloride with a 5% when EPG \geq 1,500 (Chemical); and group 3, each animal received 3g of pellets without fungi for each 10 kg of body weight, twice a week for six months, serving as Control group. The animals were weighed and it was made EPG, larval cultures, PCV and in the animals every 15 days. Monthly, samples of grass from each paddock were collected for quantification of L3/ kg D. M.

Results: The groups EPG mean began to statistically differ from day 30 (p<0.05). At day 180, the EPG mean of Fungus + Chemical group was 480, Chemical group was 1320 and Control group was 2340. The Fungus + Chemical group required less deworming with Levamisole

Hydrochloride 5% when OPG \geq 1500 (p<0.05), only eight, while Chemical group required 17. *Haemonchus* sp. was the most prevalent helminth gender in all larval culture. The Fungus + Chemical group showed superiority in PCV values throughout the experiment (p<0.05). There was a significant reduction (p< 0.05) in the recovery of L3 on pasture on the Fungus + Chemical paddock from day 30, peaking at day 180 with an average of 800 L3/ kg D.M.

Conclusions: In conclusion, the use of pellets of *D. flagrans* associated with strategic anthelmintic treatment with Levamisole Hydrochloride 5% was effective in controlling gastrointestinal nematodiosis of sheep kept in irrigated pasture in the Brazilian semiarid region.

Comments: Keywords: integrated control, nematophagous fungi, *Haemonchus* sp., farming sheep.

Small Ruminants

P01-001-101

Assessing the effect of a reduced mulesing wound size on breech strike risk parameters and wound healing in Merino weaners

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Objectives: The Code of Practice for the Welfare of Sheep states that mulesing should be performed with the minimum number of cuts suitable to the individual for flystrike protection. With the move towards a plainer bodied flock, assessment of the necessity for the current modified 'V' standard mules is required. The objective of the current study was to compare the effect of a smaller, conservative mules to the modified 'V' standard mules on wound healing and breech strike risk parameters. The outcomes of this study will also provide a scientific basis to refine industry best standards.

Materials and Methods: Two separate trials were performed on Merino ewe weaners (6-8months) in the Southern Tablelands of NSW, Australia. Animals were randomly assigned to one of two treatment groups; industry standard modified 'V' mules (MOD, 6-8 cuts) (n=100) and the conservative (CONS, 4 cuts) (n=100). In both trials, sheep were weighed and scored for key breech strike risk parameters (breech wrinkle, BRWR and perineal bare area or breech cover, BCOV) prior to and following mulesing treatment. Wounds were photographed at Day 0 and Day 28 relative to mulesing and analysed using digital planimetric software (PictZar) to obtain measurements of wound surface area (WSA, cm²), WSA to body surface area ratio (WSA:BSA) and contraction rates as an indication of healing. Tissue removed during mulesing was weighed (g).

Results: There was a significant treatment x farm interaction for weight of tissue removed (P = 0.018). The mean weight of tissue removed for the CONS treatment was significantly smaller than the MOD at both Farm 1 (85.14 \pm 1.72 g vs 113.73 \pm 1.72 g) and Farm 2 (46.17 \pm 1.73 g vs 66.54 \pm 1.75 g). There was a significant treatment x time interaction for WSA (P < 0.001). The MOD treatment resulted in a larger WSA than CONS on both Day 0 and Day 28. There was a significant reduction in WSA from Day 0 to Day 28 for both CONS (87.62 \pm 1.25 cm² vs 17.25 \pm 1.25 cm²) and MOD (150.82 \pm 1.25 cm² vs 29.53 \pm 1.26 cm²) treatments. Both treatments resulted in an 80% reduction in WSA over the 28 days. There was a significant interaction of time x treatment for BRWR (P < 0.001). Both the CONS and MOD treatments resulted in a significant reduction in BRWR (- 0.57 vs - 0.92) and BCOV (- 1.67

vs - 2.11) ($P < 0.001$). MOD treatment had significantly lower BRWR and BCOV scores than the CONS treatment ($P < 0.001$) on both farms.

Conclusions: The findings from this study provide new information and are supportive of the use of the conservative mules over a range of flock phenotypes. A conservative mulesing wound results in less mulesing cuts, less tissue removed and faster wound healing, presenting a welfare benefit to sheep undergoing the procedure. Furthermore, this method still resulted in significant improvement in breech strike risk parameters, albeit not to the same degree as the MOD method. Follow up of these sheep in flystrike seasons is important to validate the protection provided by the CONS vs MOD mulesing methods.

Small Ruminants

P01-001-102

Study Of Genetic Resources: Morphological Characteristics And Goats Morphostructural Native Community Of Cebadillas False Pen, Maravatío Michoacan

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Objectives: The objective of this work was to study phenotypically (morphology and morfoestructuralmente) native goats (Creole) located in Town of Cebadillas Corral Falso is located in the Municipality of Maravatío in the State of Michoacán de Ocampo. Cebadillas Corral Falso is 2320 meters above sea level

Materials and Methods: 110 adult animals (60 females and 50 males) from 12 deferent production units were selected and determined (by visual assessment) morphological variants TO Ears size (small, medium and large) to your ears, DO (upright, horizontal or falls), consistency of the ears, CO (rigid or pending), type of udder, TU (globosa, baggy or palillera), e-nipples, DP (parallel or divergent); and morfo-structural variables head width (ACF), head length (LCF); face length (LR), height at the withers (ACR), thoracic perimeter (PT), longitudinal diameter (DL), dorsoesternal diameter (DD), distance between horns (DC), rump length (LG), width rump (AG), width between legs (AEA), height at moths (AP) and perimeter cane (PC). For morfo-structural determinations inextensible tape measure from 1 to 150 cm and 1 cm capacity precision used to measure perimeters they were used.

Results: Morphologically it shows that there is a predominance of large ears (38.3 p.100 p.100 in females and 44 males). In relation to the direction and consistency of the ears, floppy ears males and females as well, mainly pendulous in males and females predominate pendulum. The main way the udder is globosa (53.3 p.100) although there are baggy udders (28.3 p.100) and palilleiras (18.3 p.100), forms which in turn influence the direction of the nipples (56.6 p .100 43.3 parallel and divergent p.100). The evidence for sex differences reveal a marked sexual dimorphism for morfo-structural, especially with regard to the formation of appeal of the cross, cane perimeter, thoracic perimeter and longitudinal diameter characters. Morpho-structurally no significant differences between sexes

Conclusions: Regarding the morphological characters Creole goats

has large ears, fallen and tilting consistency in both males and females. In this case the sexual dimorphism is mainly determined by the shape of the croup, the height at the withers, the width of the head and around the perimeter of cane.

Small Ruminants

P01-001-103

Effect of feeding silages from plant raw materials on the profile of fatty acids, cholesterol, and vitamins A and E in lamb meat

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Objectives: The type of silage given to animals may influence the quality of their end products. Inclusion of red clover into the lambs' diet in the form of both green forage and silage had a beneficial effect on the fatty acid profile in intramuscular fat because it increased the contribution of polyunsaturated fatty acids (PUFA). Feeding lambs with different silages induced changes in fatty acids metabolism in the rumen that may derive from differences in the extent of PUFAs biohydrogenation. The aim of the study was to compare selected traits characterizing the health quality of meat lambs fed silages produced from red clover, alfalfa and grass.

Materials and Methods: The experimental materials were 24 single ram lambs of the Polish Longwool Sheep variety Kamieniecka weaned at the age of 70 days. There were divided into 3 equal groups that were fed ad libitum experimental silages prepared from the second cut of red clover (RC), alfalfa (ALF) and grass (GR). The initial mean body weights of lambs were comparable in all groups: RC – 22.63kg, ALF – 22.42kg, GR- 22.71kg. The specific fattening period spanned for 60 days. All animals were placed in metabolic cages, and were fed only experimental silages ad libitum. Silage samples (1.0kg) were collected once before experiment and 3 times during the experiment. In the samples of silage the fatty acid composition was determined using Gas Chromatography. The ram lambs were slaughtered at the following mean body weights in groups: RC-33.66kg, ALF-34.69kg, GR- 31.75kg. Samples for meat analysis (100g) were collected from the musculus longissimus lumborum. In the meat following parameters were determined: cholesterol content (enzymatic method), vitamin A and E levels (high – performance liquid chromatography – HPLC method). Additionally the intramuscular fat content was determined by the Soxhlet method. The results were processed by one-way ANOVA test, and the significance of differences between groups was verified with Duncan's test using Statistica 9.0 software.

Results: The analyzed silages did not differ significantly in the content of saturated fatty acids. The silage from alfalfa was characterized by the lowest content of monounsaturated fatty acids (C18:1 – oleic and C14:1 – myristic) and the highest content of PUFA acids. The lowest content of PUFA acids was observed in the grass silage. The highest content of vitamin E in lamb meat was observed in the group fed grass silage. The highest content of fat was assayed in the meat of RC lambs (fed the silage from red clover).

The intramuscular fat of the lambs fed the grass silage was characterized by the highest contribution of C12:0 (lauric) and C14:0 (myristic) acids and the lowest content of C18:0 (stearic) acid. In the same group of lambs the highest content of C14:1 (myristoleic) acid and the lowest content of C18:3 (alpha-linolenic) and C20:2 (eicosanoic) acids were observed. The content of C18:3 and C20:2 acids was the highest in the fat of RC lambs.

Conclusions: The analysis of traits characterizing the health quality of lamb meat points to different effect of the silages administered to lambs in the fattening period. The silage from red clover was found to enhance intramuscular fat deposition in ram lambs, especially when compared to the silage from grass. The results of our study indicate that the species of plants used to prepare silage exerts a significant effect on the quality of silage and its intake by lambs. As consequence, it determines nutrient absorption and health-promoting properties of lamb meat.

Small Ruminants

P01-001-104

Coadministration of nematophagous fungi in the biological control of sheep gastrointestinal nematodes in Northeastern Brazil

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Objectives: It was aimed to evaluate the coadministration of fungi *D. flagrans* and *M. thaumasium* in sodium alginate matrix on gastrointestinal nematodes of lambs and adults sheep in the semiarid region of Northeastern Brazil.

Materials and Methods: An area of 1 ha (approximately 2,5 English acres) was divided into two paddocks and two experimental groups (one in each paddock) were placed on these places. Also, each group was composed by six adult females and ten young males, providing two experimental groups (Fungus and Control group). In each group, two subgroups have been created in accordance with the animal type (Adult and Young). In Fungi group, each animal received, in the feed, 3g of the pellets (0.6 g of fungal mycelium, with 0.3g of *D. flagrans* and 0.3g of *M. thaumasium*), for each 10 kg of body weight, twice a week, for six months. In Control group, each animal received, in the feed, 3g of pellets without fungus, also for each 10 kg of body weight, twice a week, during six months.

Results: It was observed a reduction of 76 % in the Eggs per gram (EPG) of subgroup Fungi - Adults and a percentage of 83 % in the subgroup Fungi - Young, when compared to their respective Control subgroups. The groups that received these fungi needed less salvage deworming, and they presented the best PCV percentages in the days 150 and 180 ($p < 0.05$). In addition, they had the best weight gain (18.7 kg) and a significant reduction of 91.6% in the number of L3/ kg D.M. in its paddock, when compared to Control groups.

Conclusions: Finally, it is concluded that *D. flagrans* and *M. thaumasium* coadministration was effective in the control of gastrointestinal nematodes of adults and young sheep in the semiarid region of Northeastern Brazil.

Keywords: *Duddingtonia flagrans*, nematophagous fungi, *Monacrosporium thaumasium*, sheep farming

Small Ruminants

P01-001-105

Glutamate Precision Supplementation, Antral Follicular Development And The Secretion Pattern Of Lh In Adult Goats.

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Objectives: Nutritional supplementation, even without detectable increases in live weight, the “acute effect of nutrition”, can increase reproductive outcomes. The physiological signals involved are not fully elucidated, but activation of glutamatergic neurons should be implicated. Glutamate is a primary mediator of excitatory synaptic transmission in the CNS and has receptors localized in a variety of hypothalamic nuclei, some of which are critical for reproductive function. We aimed to evaluate the possible effect of acute-short term glutamate supplementation upon antral follicular development and the serum levels, pulsatility and AUC of LH in goats.

Materials and Methods: The experiment was carried-out at the Southern Goat Research Unit, UACH-URUZA, Mexico (25°NL, 1,117m) under decreased photoperiods (Nov). Adult goats (n=22) 7/8 Sannen-Alpine were randomly distributed to individual pens and divided into two experimental groups, with homogeneous live weight (LW) and body condition (BC): 1) Glutamate group (GLUT, n=10, LW=45.8±4.37 kg; 0.175 mg kg⁻¹ LW of glutamate, i.v. applied on days 1, 9, 14 and 17 post-ovulation and 2) Control group (CONT, n=12, LW=46.2±5.87 kg; received saline (0.0875 mL kg⁻¹ LW, i.v.). The basal diet considered alfalfa hay (14% CP; 1.14 Mcal kg⁻¹ mNE) and corn silage (8.1% CP; 1.62 Mcal kg⁻¹ mNE). After 2-wk adaptation period to general management and conditions, the estrous cycles were synchronized with the use of intravaginal sponges; glutamate was applied on days coincident to the emergence of the four follicular waves. The population of antral follicles (> 5 mm) was estimated on d19 (the mid-follicular phase) throughout transrectal ultrasonographic scanning (TUSS) with a 7.5 MHz linear-array transducer (Toshiba Medical Systems, Ltd, Crawley, UK) and an intensive blood sampling (6 h x 15 min) was performed the last TUSS to evaluate serum LH through RIA. Data were analyzed by a CRD-ANOVA, while separation of the means was assessed using the LSMEANS-PDIFF option of the PROC GLM. All statistical analyses considered the procedures of SAS (SAS Inst. Inc. V9.1 Cary, NC, USA). Correlation analyses between different response variables were conducted by Pearson's product-moment test; least-square means ± SEM are reported.

Results: Both LW (44.5±1.3 Kg) and BC (3.3±0.8 units) did not differ ($P > 0.26$) between treatments. In addition, the serum levels (4.94 ng mL⁻¹), pulsatility (3.8 pulses) and the area under the curve (1,804.4 units) of LH did not differ ($P > 0.05$) during the follicular phase between treatments. However, an increased antral follicular population ($P = 0.05$; 3.40 vs. 2.18) favored to the GLUT-group. Results confirmed an acute effect of the short-term supplementation with L-glutamate, stimulating the steroidogenic activity while promoting a greater development of the antral follicular population. The last, without changes in the secretion pattern of LH, considering concentration, pulsatility and area under the curve of LH. The site of glutamate action, at brain or ovarian level, cannot be deciphered without further work, but there are some interesting possibilities. In the hypothalamus, the excitatory amino acid glutamate plays a critical role in the activation of gonadotropin secretion, particularly, in the production of the preovulatory surge of

GnRH. Glutamatergic neurons involved in the control of GnRH neurons contain kisspeptin+GPR54, a molecular complex which play a crucial role as essential regulator of the gonadotropic axis. Besides, ionotropic glutamate receptors have been identified at ovarian level while glutamate itself is present in antral fluid at several stages of follicular development.

Conclusions: Results confirmed a short-term-acute effect of the precision supplementation with L-glutamate during specific windows of the estrous cycle upon follicular growth in adult goats, stimulating the steroidogenic pathway while promoting a greater development of the antral follicular population. The last physiological scenario occurred without changes in the secretion pattern of LH, considering overall concentration, pulsatility and area under the curve of LH. Even so, the precise site of glutamate action, either at hypothalamic centers or at ovarian level, remains to be deciphered; results may encompass translational applications.

Small Ruminants

P01-001-106

Genetic parameters for health traits in a multi-breed sheep population

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Objectives: The objective of the study was to quantify the genetic variation present in three key health related traits (i.e., lameness, dagginess and mastitis) and their genetic association with body-related traits (i.e., body condition score (BCS), live weight (LW), muscle depth and fat depth) in a multi-breed Irish sheep population.

Materials and Methods: Records were available on 35,282 animals (i.e., lambs and adults) from the Sheep Ireland database between the years 2009 and 2015, inclusive. Each trait was measured by trained technicians. Mastitis was measured as a binary trait (0=no mastitis; 1=mastitis). Lameness was visually assessed on a 3-point scale (0=not lame, 1=slightly lame, 2=moderately to severely lame). Dagginess, a measure of the build-up (or lack thereof) of faecal material around the hindquarter of the animal, was measured on a 5-point scale: 1=clean to 5=faecal material covering the breech area and extending down the hind legs. Live weight was recorded using a pre-calibrated (electronic) scales. Both muscle depth and fat depth were measured using ultrasound scanning. BCS was subjectively assessed on a scale of 1 (extremely emaciated) to 5 (over-fat). Animals without a known sire were excluded, as were all rams (i.e. male animals ≥ 365 days old) and ewes that had previously been used as embryo transfer donors. Variance components were estimated using linear animal mixed models. Fixed effects included in the model were the breed proportion of the animal, coefficients of heterosis and recombination, parity/age group, and the age difference from the median of each parity/age group; an additive genetic effect and residual effect were both fitted as random terms with the animal pedigree considered via a numerator relationship matrix.

Results: The heritability estimate for dagginess and lameness across all data were 0.13 (SE=0.01) and 0.07 (SE=0.01), respectively. The heritability estimates for LW and BCS were 0.28 (SE=0.02) and 0.12 (SE=0.02), respectively. Mastitis, which was only measured in ewes, had an estimated heritability of 0.04 (SE=0.04). Muscle depth and fat depth had heritability estimates of 0.05 (SE=0.01) and 0.30 (SE=0.06),

respectively and were measured only in lambs. Body related traits were all strongly phenotypically correlated; the phenotypic correlations between LW and BCS, muscle depth and fat depth were 0.52, 0.42 and 0.26 respectively. The phenotypic correlations between dagginess and BCS, LW and muscle depth were -0.09, -0.03, and -0.06, respectively. Weak genetic and phenotypic correlation existed among lameness, mastitis and dagginess. Similarly, weak genetic correlations existed between LW and dagginess, or between lameness and mastitis while the phenotypic correlations between LW and dagginess, lameness and mastitis were -0.03, -0.09 and -0.03, respectively. The genetic correlation between lameness and BCS was -0.51 (SE=0.13) while the genetic correlation between dagginess and muscle depth was -0.26 (SE=0.13). Body related traits were strongly correlated with each other; the genetic correlations between BCS and LW, muscle depth and fat depth were 0.76, 0.45 and 0.40, respectively. The genetic correlations between LW and muscle depth and LW and fat depth were both 0.75, while the correlation between muscle depth and fat depth was 0.81.

Conclusions: Ample genetic variation exists for all the health traits investigated in the current study indicate that genetic improvement in dagginess, lameness and mastitis is possible. Furthermore, knowledge of the correlations between the health and production traits indicates that it is possible to select for improved health traits without compromising on production gains.

Small Ruminants

P01-001-107

Staphylococcus aureus Associated With Subclinical Mastitis From Sheep Meat Flocks From Small Production Units In Two Regions Of The State Of Mexico.

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Objectives: The objective was to determine the frequency of subclinical mastitis and associated pathogens with mammary gland infection in sheep meat flocks from small production units in two regions of the State of Mexico.

Materials and Methods: A longitudinal descriptive study was conducted using a non-probabilistic convenience sampling in sheep production areas in the central-eastern and northeastern region of the state of Mexico in smallholder semi-intensive sheep farms where the following meat breeds were found: Suffolk, Hampshire, Corriedale and hybrids with prevalent creole flocks in the north-eastern region. 625 composite samples from the udder of lactating ewes were collected; lambs were separated one hour prior to hand milk. Milk was obtained as a single composite sample in sterile plastic tubes, kept at 4 °C until microbiological tests were run. Subclinical mastitis was detected using California test. Reactions were interpreted as negative, trace, one, two and three. For microbial isolation milk was homogenized at 25 °C, and 0.01 ml of milk was inoculated on blood and MacConkey agar plates, and incubated at 37 °C for 18 to 24 hours. Colony forming units were identified by Gram staining, coagulase, Voges-Proskauer, catalase, oxidase, triple sugar iron (TSI), and motility tests. Final identification was performed using

ApiStaph and API20E system. The isolated groups of pathogens were classified as contagious (*S. aureus* and *St. agalactiae*), environmental (*Escherichia coli*, *Klebsiella* spp, *Enterobacter* spp, *Streptococcus* none *agalactiae*), and as minor (coagulase negative *Staphylococcus*). Results were evaluated by testing estimated proportions ($p < 0.05$). Analysis of the results was made through the frequencies of isolation observed, subclinical mastitis and regional distribution in sheep flock units.

Results: Subclinical mastitis frequency was 43%, and 0.4% for sheep with clinical mastitis, different in all sampled regions ($p < 0.001$). Isolated pathogens were (%): *Staphylococcus aureus* 36.2, *Staphylococcus coagulasa* negativos 12.5, *Streptococcus* spp. 6.5%, *M. hemolítica* 5.4., *Klebsiella* spp 2.8, *Pasteurella multocida* 0.2, *γ* *Arcanobacterium pyogenes* 0.4. The general isolation rate was high in the different regions with no associations according to the breed ($p < 0.05$). Subclinical mastitis is mainly related with *Staphylococcus aureus*, negative coagulase *Staphylococcus*, and in less proportion *Streptococcus* spp and other environmental pathogens. Clinical mastitis cases were directly related with *S. aureus*, *Streptococcus* spp., and *A. pyogenes*.

Conclusions: We conclude that *S. aureus* and coagulase-negative *Staphylococcus* showed a wide distribution in sheep flocks compromising the health of the mammary gland and its capability to produce milk for rearing of lambs, therefore affecting sheep meat production systems from smallholder sheep farms.

Small Ruminants

P01-001-108

Patterns of heat tolerance in different sheep breeds in Brazil

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Objectives: Physiological mechanisms which limit and adjust cold and heat tolerance are regaining interest due to global warming, therefore this study aimed to determine regions of Brazil suitable for sheep breeds with differing in heat adaptation.

Materials and Methods: Data were collected on 80 sheep from 11 breeds using a total of 1077 observations. Breeds included Australian Merino, Polwarth/Ideal, Corriedale, Romney Marsh and Crioula for wool sheep. Short wool sheep included Hampshire Down, Texel, Ile de France and Suffolk, while hair sheep included Santa Inês and Dorper. All sheep were adult and non-lactating. Eye temperatures (T_{eye}) were taken in both eyes using an infrared camera (FLIR ®) and average temperature calculated. The respiratory rate (RR) was assessed by movement of the chest wall and abdomen and counted. During the evaluation, the animals were at rest and were not feeding or ruminating. The air temperature (T_{air}) and relative humidity (RH) of the housing were evaluated at several points using a digital thermo-hygrometer. Measurements of the shed conditions - T_{air} and RH - and the physiological parameters of animals - T_{eye} and RR - were taken three times a day: in the morning between 7 and 9; afternoon between 13 and 15 and night between 18 and 20 hours. For data collection there was no interference in the behavior and physiological responses of animals due to management as the sheep were not handled or disturbed during the experiment. The temperature humidity index (THI) was calculated from temperature and humidity data. Data were analysed, using broken line regression to determine THI, T_{air} and RH limits by breed for respiration rate and T_{eye} . These inflexion points were then used to determine regions suitable for sheep rearing by

breed, using mean THI by municipality and spatialized using ARCGIS ®.

Results: The mean THI (THI_m) was 20.30, ranging from 14.02 to 31.44. There was an increase in RR and T_{eye} with T_{air} and THI and a decrease in RR with an increase in RH. Correlations between THI and THI_m were 0.99. The inflexion point for RR indicates the point (THI, T_{air} or RH) that the animal changes its respiration to compensate for environmental changes, while for T_{eye} inflexion point is the value where physiological mechanisms no longer can maintain the core temperature under control. The hair breeds tended to have faster reactions to increases in THI and T_{air} , as expected. Variation in responses for Dorper, Santa Ines and Ile de France were more varied and in general lower. T_{eye} were highest in Romney, Merino, Ideal and Suffolk, while Crioula showed lower T_{eye} , although it used respiration at a lower temperature managed to maintain T_{eye} at a low level. The Southern region is the region most suitable for all breeds of sheep. Some municipalities in the Southern Region are unsuitable even for rearing Suffolk or Texel breeds, while the Santa Ines can be reared in almost all environments. However, there are some regions where sheep cannot be reared. There is a band of municipalities running from the center-west to the northeast of the country where many sheep breeds would be under stress on an average day. A large portion of the northern region is covered by forest and therefore, although adequate environmental conditions exist, is not used for sheep production.

Conclusions: To determine differing regions of Brazil for sheep breeds in heat adaptation is required to re-evaluate thermal limits, to adjust cold and heat tolerance, for sheep breeding, especially in Brazilian hair sheep.

Small Ruminants

P01-001-109

A novel ELISA for the detection of caseous lymphadenitis in United States goat herds: Test validation and application in a field setting

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Objectives: Commercially available diagnostics for caseous lymphadenitis (CL) in the United States include bacterial culture and Synergistic Hemolysis Inhibition (SHI) serological testing, whereas ELISA testing is used more extensively outside of the US. ELISA testing may be more economical and because most US diagnostic laboratories have ELISA capabilities, could become more widely available. The objectives of this study were to validate a CL ELISA test commercially available in Europe, then use the ELISA in subsequent field testing to determine CL prevalence in Midwestern United States dairy goat herds.

Materials and Methods: For test validation, goat serum from 78 positive controls and 138 negative controls were collected. Serum samples were tested using ELISA plates (Elitest CLA, MV Diagnostics, Edinburgh, UK), per kit instructions and SHI testing performed at an accredited diagnostic laboratory. Positive and negative results were recorded and compared to known disease status. Sensitivity and specificity of each test was calculated.

For field application of the ELISA test, serum samples (n=543) were collected from 4 dairy goat operations in Kalona, Iowa, US. Herd 1 (HD1)

consisted of 172 head of goats (age: mean=33.3m, median=18m); HD2=121 head (age: mean=45m, median=42m), HD3=122 head (age: mean=63.3m, median=63m), and HD4=128 head (age: mean=59.4m, median=54m). Any goats with abscess formation were also collected for culture. Prevalence of CL was determined for each herd, and ELISA test results were subsequently compared to culture results.

Results: All 138 negative status goats were seronegative with the ELISA and a subset of 67 negative status goats were also seronegative with the SHI test. 9/78 goats that were culture positive were negative on the ELISA (11.5% false negative), and 4/78 culture positive goats were negative with the SHI test (5.13% false negative). The sensitivity and specificity for the ELISA was 88.46% and 100%, respectively. The sensitivity and specificity for the SHI test was 94.87% and 100%, respectively. Agreement between the two serologic tests was evaluated using Kappa Statistic at 0.90 (95% CI: 0.83-0.97).

Using the ELISA in a commercial setting, the total seroprevalence of CL in herds that had known infections was 77.8%, and the prevalence of culture positive goats was 5%. The prevalence within each of the four herds varied; prevalence in HD1=65.7% (113/172), HD2=92.6% (112/121), HD3=85.2% (104/122), and HD4=77.34% (99/128). When goats from these herds that were exhibiting clinical signs were sampled for culture, 26/27 (96%) culture positive goats were also positive with the ELISA.

Conclusions: In this population, either the ELISA or SHI test could be used to achieve similar results and sensitivity and specificity were high for both tests. However, variations between sensitivity and specificity in previous studies indicate the need case-controlled studies over a wider geographic population. The field study showed that culling all seropositive animals could nearly depopulate all herds, and negative status could not be guaranteed. Therefore, in high prevalence herds, clearer understanding of risk factors for abscess formation and a stronger focus on management rather than eradication needs to be evaluated.

Small Ruminants

P01-001-110

Caprine Arthritis Encephalitis (CAE) and Maedi-Visna by AGID and PCR in goat and sheep herds from State of Rio de Janeiro, Brazil

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Objectives: Caprine Arthritis Encephalitis (CAE) and Maedi-Visna (MV) are infectious multisystemic diseases of chronic nature, caused by Lentivirus group, which determines economic importance to sheep and goat industry due to production losses and trade barriers. Both diseases are regulated by the Sheep and Goat Health National Program of the Ministry of Agriculture, Livestock and Supply, Brazil. In order to investigate these lentiviruses, sheep and goat blood samples were collected in the period of 2013 to 2015, in seven farms in the state of Rio de Janeiro, Brazil.

Materials and Methods: A total of 242 sheep, distributed in farms A (n = 95), B (n = 36), C (n = 65), D (n = 30) and E (n = 16) had serum samples tested for Maedi-Visna and CAE by Agarose Gel Immunodiffusion (AGID). Farms A, B and C adopted the semi-intensive system of

production, while the farm D had an intensive system for blood products and E, belonging to the Brazilian Army (IBEX), sheep found themselves confined. Also, 57 serum samples from goats were collected from two farms in the state of Rio de Janeiro (29 and 28 sera from F and G establishments, respectively). These properties had adopted systems of semi-intensive and intensive breeding respectively. For diagnostic of Lentivirus by Nested-PCR, blood samples of 26 sheep from farm A and 11 goats (farm G) were collected with EDTA in the same period described above. For AGID was used a commercial kit (Biovetech®), following the manufacturer's instructions. In the first stage of Nested-PCR (Barlough et al., 1994), 34µL of ultra-pure water, 5µL 10x buffer, 2 µL 50 mM MgCl₂, 2 µL 10mM dNTP, 1.5 µL of each primer and 0.2 µL of Taq DNA polymerase 5U were used for obtaining an "amplicon" of 297bp. Then, in the second stage, 36µL of ultra-pure water, 5µL 10x buffer, 2 µL 50 mM MgCl₂, 2 µL 10mM dNTP, 1.5 µL of each primer and 1 µL of Taq polymerase 5U were used to the second Nested-PCR amplification, generating an "amplicon" of 185bp. The amplifications were obtained in a thermocycler programmed. The amplified products were subjected to agarose gel electrophoresis and visualized under UV light at transilluminator.

Results: We observed that 2.48% (06/242) sera sheep were reactive for Maedi-Visna, being obtained from two farms (A and C). While for CAE, 2.89% (07/242) of the tested sheep sera were reactive, all from one establishment (A). According to the literature, it is expected higher prevalence of lentiviruses in intensive farming systems, but there was no positive animal on the premises D and E. Facilities A and C showed greater flow of buying and selling animals, and probably this rotation without proper sanitary control, had worked as a potential risk factor for the spread of the virus.

In addition, four samples of sheep sera were reactive for CAE, as for Maedi-visna by AGID, which can be explained by the mixed infection or cross-reaction, necessitating a greater in-depth study. For this elucidation, a sequence to typify Lentivirus can be identified after completion of PCR. Already, in two herds of goats, the positivity of animals tested for CAE, by AGID, was 29.82% (17/57). The results of PCR in sheep samples demonstrated that 11.54% (3/26) were positive. Although AGID and ELISA constitute the tests recommended by OIE, three sheep samples were positive by PCR, but, at the same time, none of them were reactive by AGID for CAE and Maedi-Visna. This fact can be justified by the absence of seroconversion in the initial phase of infection and / or the greater sensitivity of PCR. Besides that, 18.18% (2/11) of goats were positive by PCR. The use of PCR together AGID improved diagnostic efficacy, particularly in cases of animals with serology negative or doubtful.

Conclusions: In this study, it was found reactive samples by AGID for MV and CAE, both in sheep and goats herds; as well as mixed infection or cross-reaction, in the State of Rio de Janeiro. It was evident the importance of epidemiological monitoring for obtaining the incidence of these lentiviruses. Additionally, the use in conjunction of AGID and PCR improved the diagnostic accuracy, especially in cases of doubtful or negative serology.

Small Ruminants

P01-001-112

Reproductive Performance Of Nulliparous Anovulatory Ewes Exposed To Glutamate And Or Testosterone Treated Rams During Increased Photoperiods In Mexico

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Objectives: The aim of this study was to evaluate the possible effect of glutamate and(or) testosterone treated males upon the male sexual behavior and the reproductive outcomes of nulliparous Dorper ewes once exposed to such males during the natural spring anestrus season in Northern Mexico (25° N, 103° W).

Materials and Methods: 8 Dorper rams, and 55 nulliparous-anovulatory ewes were used. Two transrectal ultrasound screenings were performed on days -14 and -7 prior the experimental breeding to confirm absence of corpora lutea. Thereafter, prior exposure to males, each ewe received a total of 60 mg i.m. of progesterone divided in 3 doses (20 mg each) on days -6, -4 and -2 prior to the male effect, in order to avoid short cycles and to promote ovulation accompanied by standing estrus. Both ewes and rams were divided into four experimental groups, 1). Glutamate Group (GG, 14 ewes exposed to two rams treated 7 mg kg⁻¹ BW of L-Glutamate i.m. every 4d x 30d; 2). Testosterone Group (TG, 14 ewes exposed to two rams treated with 0.5 ml saline every 3d x 15d + 25 mg of testosterone i.m. every 3d x 15d); 3). Glutamate + Testosterone Group, (GTG, 14 ewes + two rams treated with 7 mg kg⁻¹ BW of L-Glutamate every 4d x 30d + 25 mg of testosterone i.m. every 3d x 15d, the last 15d) and 4). Control Group (CONT, 13 ewes exposed to two rams treated with 0.5 mL of saline i.m. every 4d x 30d). Each group was placed at different pen, at a distance of 100 m among them. The frequency of the appetitive sexual behaviors (ASB) and consummatory sexual behavior (CSB) were daily recorded during 2-h x 2d, and from the first day of contact with the males, reproductive activity from anovulatory ewes exposed to the male effect, the onset of estrus was recorded. Thereafter, the embryo implantation rate was determined on d-45 from male exposure. Data were analyzed with X², while onset of estrus were analyzed using t test.

Results: Regarding the ASB, the frequency of behaviors of the GGT, GG, CONT and TG groups were 43 vs 29, 18, 11%, respectively; differences (P<0.05) were observed only between TG and CONT. With respect to the CSB, the best response was depicted by the GG and GTG (32% each; P>0.05), followed by the GC (20%) with the lowest response depicted by the TG (3%; P<0.05). When evaluating the female response upon exposition to the different treated males, those ewes exposed to the GG-males depicted the best reproductive response since they showed not only the shortest interval to estrus (38.6±2.6 h) but also the highest estrus (100%) and ovulatory (100%) rates as well as the highest embryonic implantation rate (100%).

Conclusions: Such ovarian and reproductive responses depicted by these nulliparous Dorper ewes during the natural anestrus season while exposed to males treated with glutamate (GG) and glutamate + testosterone (GTG) are of paramount and potential importance to promote the sustainability. The last being of paramount importance to different animal production system depicting reproductive seasonality.

Small Ruminants

P01-001-113

Nebulization Therapy In Small Ruminants, 21 Cases (2000-2015)

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Objectives: The objectives of this study were to identify the agents and dosages utilized for nebulization therapy in small ruminants at a university teaching hospital. Additional objectives were to describe outcomes vs non-nebulized cases and report any complications arising from therapy.

Materials and Methods: Medical records of small ruminants presented to the University of California-Davis Veterinary Medical Teaching Hospital (VMTH) between January 1, 2000 and March 1, 2015 were evaluated for this study. Cases that had undergone nebulization therapy were selected.

A group of control cases of small ruminants that were not nebulized with a diagnosis of pneumonia were collected from the same search period for the same institution. A logistic regression was used to compare outcome between groups with outcome being survival to discharge from the hospital, among other treatment variables.

Results: Fourteen goats and 7 sheep that underwent nebulization were identified, all treated for some form of respiratory disease. Nebulized drugs included antimicrobials, mucolytics, and bronchodilators. In goats, the most commonly nebulized drug was ceftiofur (n=7). Dosages ranged from 1 to 14.5 mg/kg (mean 6.8 mg/kg). In sheep, the most commonly nebulized drug was ceftiofur (n=4). Two goats exhibited respiratory distress during nebulization.

Preliminary regression analysis demonstrated a negative outcome for patients that did not undergo nebulization.

Conclusions: Nebulization appears to be a minimally invasive adjunct for treatment of small ruminant respiratory disease. Practitioners should be aware of non-antimicrobial therapies for treating respiratory disease in small ruminants such as mucolytics and bronchodilators. Dosages higher than previously reported for calves (ceftiofur, 1 mg/kg) may be clinically useful for small ruminants with respiratory disease. Extralabel drug use should be considered when utilizing nebulization therapy. More research in a controlled setting is needed to further evaluate efficacy of nebulization therapies in the treatment of in small ruminants with respiratory disease.

Small Ruminants

P01-001-114

Acute phase proteins profile in Anglo Nubian goats fed with different fat sources and submitted to laparoscopic ovum pick-up

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Objectives: There is a lack of information about the effects of the diet fat source on goat reproduction and acute phase proteins (APP) response. The aim of the present study was evaluate the acute phase proteins profile in eighteen goats fed with different fat sources and submitted to twice-laparoscopic ovum pick-up (LOPU).

Materials and Methods: Eighteen Anglo-Nubian goats were randomly divided into 3 groups based on their diets with different fat sources [soy oil (S, n=6), linseed (L, n=6) and Megalac® (M, n=6)], formulated with 40% concentrate, 60% corn silage and 4% ether extract (in dry matter) from the fat source. The animals were submitted to 15 days adaptation period and, then, an experimental period of 77 days. The LOPU was performed after 36 hours of an ovarian superstimulation protocol [FSH (80mg) + eCG (300IU)], on Day 42 (LOPU1) and Day 70 (LOPU2) of the trial. The blood samples were taken just before each LOPU (D42 and D70), 24 hours (D43 and D71), 72 hours (D45 and D73), five days (D47 and D75) and seven days (D77) after LOPU. The fraction of proteins were obtained by SDS-PAGE. Data were analyzed by ANOVA for repeated measures with Tukey post-hoc test ($p \leq 0.05$) using software R. There were interaction between groups and time and the results are expressed by means \pm sd and capital letters after meaning indicate difference between groups and small letter difference between times. Were found between 16 to 18 proteins and for this study ceruloplasmine (CER, mg/dL) and haptoglobine (HAPT, mg/dL) were elected as relevant.

Results: The CER results on days D42, D43, D45, D47, D70, D71, D73, D75 and D77 of S group are 34.4 ± 13.4 Aa; 26.2 ± 8.95 Aa; 42.7 ± 21.3 Aa; 60.7 ± 23.4 Aa; 35.4 ± 10.2 Aa; 38.9 ± 23.0 Aa; 46.7 ± 27.0 Aa; 34.2 ± 25.4 Aa; 29.1 ± 18.6 Ba; of group L are 56.0 ± 37.5 Aab; 47.0 ± 24.8 Aab; 74.0 ± 50.9 Aa; 47.9 ± 12.2 Aab; 38.0 ± 14.8 Aab; 35.0 ± 9.66 Aab; 29.3 ± 21.9 Ab; 53.9 ± 19.6 Aab and 54.3 ± 21.5 Abab, and in M group are 38.4 ± 17.9 Aa; 47.3 ± 14.5 Aa; 69.6 ± 44.0 Aa; 42.7 ± 25.7 Aa; 52.8 ± 38.5 Aa; 40.5 ± 22.4 Aa; 46.5 ± 10.7 Aa; 43.9 ± 20.6 Aa and 64.3 ± 21.6 Aa. The HAPT results on days D42, D43, D45, D47, D70, D71, D73, D75 and D77 of S group are 16.3 ± 20.6 Bc; 76.1 ± 24.5 Aa; 116 ± 102 Aab; 87.1 ± 73.4 Aab; 20.9 ± 13.6 Abc; 140 ± 123 Aa; 183 ± 176 Aa; 137 ± 174 Aa and 152 ± 218 Aa; of group L are 78.7 ± 64.9 Aab; 124 ± 74.8 Aa; 168 ± 85.7 Aa; 105 ± 78.0 Aa; 19.3 ± 5.03 Ab; 108 ± 34.4 Aa; 148 ± 149 Aa; 176 ± 202 Aa and 136 ± 169 Aab, and of M group are 31.9 ± 25.9 Abc; 106 ± 39.8 Aab; 128 ± 78.8 Aab; 68.2 ± 50.5 Aabc; 46.9 ± 49.4 ; Abc; 258 ± 318 Aa; 89.6 ± 49.9 Aabc; 54.9 ± 38.9 Aabc and 26.8 ± 13.5 Ac. The values of CER were different between moments only in group L with the highest values on D77 and D43 and lowest on D75. The values of HAPT were different between groups only on D42 with the highest values measured on L group and the lowest on C group, in C group the highest value were on D75 and lowest on D42, on L group the biggest value were on D43 and the smaller on D77, in group M the biggest values were on D71 and smaller on D77.

Conclusions: According to our results CER and HAPT were higher on L group and despite what was reported in monogastric animals and humans the enrichment of diet with polyunsaturated fat acids (PUFA) goats fed with higher levels of pufa have a bigger APP response. There might be an influence from the source of dietary fat on APP profile of goats and more studies are necessary to explore these effects.

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Small Ruminants

P01-001-115

Reproductive Tract Surgeries in Goats under General Inhalational Anaesthesia

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Objectives: Goat (*Capra hircus*) is gaining acceptance as an established model for research about ruminant reproductive tract physiology. Goats can be gentle, easy to handle and transport, intelligent, affectionate, friendly and clean. The study of oviduct contractility (isthmus and ampulla) near ovulation time in females is still not enough nevertheless overall understanding is vastly important in clinical practice especially in seasonally polyoestral animals. The aim of the study was to record myoelectric activity of the oviduct and uterus in goats following induced oestrus with different synchronisation protocols during breeding season.

Materials and Methods: The 30 polish white landrace goats were enrolled into the study. The elective, surgical, procedure under general inhalational anesthesia (sevoflurane in oxygene) was performed in all animals. The general inhalational anesthesia (sevoflurane in oxygen; Sevoflurane Baxter) with endotracheal intubation following intramuscular premedication (midanium 0,4 mg/kg; Midanium 5mg/ml Polfa Warszawa S.A., ketamine 4mg/kg; Bioketan 50 ml Vetoquinol Biowet PL) and intravenous induction (propofol 4mg/kg; Scanofol 10mg/ml ScanVet Poland) was performed due to the left flank laparotomy. The silicone base silver bipolar electrodes were adapted to be sutured on oviduct and uterine horn. Goats were surgically fitted with TL10M3-D70-EEE (DSI, St. Paul, MN, USA) implants positioned between the abdominal muscles, and 3 silicone electrodes sutured on the left or right oviduct (bulb and isthmus) and the corresponding uterus horn. The myoelectric activity was recorded using the DL10 analog output (DSI) coupled to PowerLab (ADInstruments, Melbourne, Australia) and PC computer. The postoperative antibiotic therapy with amoxicillin 1ml/10kg (Betamox L.A 150mg/ml; ScanVet Poland) in combination with postoperative analgesia meloxicam 0,5 mg/kg (Metacam 5mg/ml; Boehringer Ingelheim Vetmedica) was proceed for 5 days. Goats after the research underwent euthanasia with intravenous injection of phentobarbital 0,3- 0,6 ml/kg (Morbital; Biowet Puławy) to collect tissues for further histopathology examination and to obtain uterine and ovarian explants.

Results: The maintenance of general anaesthesia (sevoflurane in oxygene) was smooth and easier to control compared with general anaesthesia maintenance with isoflurane in oxygene. No complications like heart arrhythmias, respiratory depression and anaphylactic reaction were observed during surgery. The recovery process after endotracheal tube removal was much faster (5 min- standing position) without complication in intensive care room for large animals compared with recovery process after isoflurane administration (15 min). The back to consciousness after sevoflurane anaesthesia is faster than after isoflurane anaesthesia which is preferably especially in ruminants.

Conclusions: General inhalational anesthesia (sevoflurane in oxygene) allows for optimal however safely, painless management of the highly complicated surgeries on reproductive tract in goat model. Furthermore, the medical sciences state of art progress affords application of the latest anaesthetic drugs and techniques allowing to minimize pain and distress in animals.

Small Ruminants

P01-001-116

Hematological and biochemical evaluation of goat whole blood stored in CPDA-1 and CPD/SAG-M bags.

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Objectives: Considering the variety of blood bags and the different benefits of the substances used, the implementation of different conservation systems in veterinary transfusion medicine should be carefully evaluated. It is important to know which are the alterations that occurring in blood during storage in different animal species. Thus, we aimed to evaluate the hematological and biochemical changes of whole blood of goats stored in CPDA-1 and CPD/SAG-M bags.

Materials and Methods: Seven male, adult, mixed-breed goats were used, weighing an average of 62kg. From each animal 900 ml of whole blood was collected directly into CPDA-1 and CPD/SAG-M plastic bags (450 ml of blood in each bag). Bags were stored for 42 days at an average temperature of 3°C and were daily manually mixed for 5 minutes. Blood samples were taken from the bags at seven moments: T0 (baseline: immediately after collection of blood), T7d, T14d, T21d, T28d, T35d and T42d (seven, 14, 21, 28, 35 and 42 days after collection). In every moment, hematological (number of erythrocytes, leukocytes, packed cell volume and total plasma hemoglobin, degree of hemolysis and mean corpuscular volume), biochemical (glucose, lactate, sodium, potassium and total protein.) and microbiological (incubation in Agar blood) analyses were performed.

Results: The difference between the volume of diluent contained in bags made it difficult to compare results. In both bags there was an increase ($p < 0.05$) in plasma hemoglobin, degree of hemolysis and lactate during the storage period and a reduction of total hemoglobin and pH. No alterations were observed in the number of erythrocytes, glucose, sodium and potassium when compared to baseline. The CPD/SAG-M bag showed lower values of erythrocytes, leukocytes, packed cell volume, total hemoglobin and sodium when compared with CPDA-1, probably due to the dilution, but presented a higher ($p < 0.05$) degree of hemolysis and plasma hemoglobin.

Conclusions: The changes observed in goat whole blood stored for 42 days in CPDA-1 and CPD/SAG-M, were not sufficient to invalidate the use of blood stored for transfusion purpose, however, considering our results and the lower price of CPDA-1 bags, these are most advantageous for the storage of goat blood.

Objectives: The caseous lymphadenitis is a disease caused by a gram positive bacteria *Corynebacterium pseudotuberculosis*, encountered in mucous membranes and in the soil, which can remain viable for several months. After contamination by oral, respiratory or skin wound, the bacteria are phagocytosed by neutrophils and macrophages and sequestered to regional lymph nodes, which form pyogranulomas. The objective of this paper is to report a case of brain abscess caused by *Corynebacterium pseudotuberculosis*, not reported in the consulted literature.

Materials and Methods: A male goat breed Boer three-year-old has been evaluated with history of ataxia, apathy, dyspnea and progressive weight loss for 45 days. On clinical examination it was observed dyspnea, ataxia of the hind limbs, enlarged lymph nodes, rumen hypomotility, hypermetria and proprioceptive deficit in the hind limbs, especially in the right side. The hemogram revealed increased fibrinogen (1000g / dL) and inversion of the ratio of neutrophil leucocytes and (3: 1). He was suspected of bacteremia by *C. pseudotuberculosis* due to lung infection, for which it was prescribed florfenicol for 15 days. After 18 days, the patient was more ataxic, walked around in a circle, had lip and eyelid ptoses and mydriasis in the right side of the face, and seizures in 20min intervals. Due to the worsening of clinical signs, the animal was euthanized.

Results: At necropsy was observed pus in prescapular, mandibular lymph nodes and increased mediastinal lymph nodes, pulmonary emphysema and pericardial adhesion in cranial lung lobes. In the central nervous system was found an abscess of 5cm diameter containing pus close to the occipital cortex and left cortex temporal. Histologically, the white matter of the brain had extensive focal areas of cell necrosis, with intense increase of mononuclear cells mostly associated with foamy macrophages and astrocytes in astrogliosis process, as well as rare presence of giant cells. Pus found inside the brain underwent microbiological analysis, which was isolated *Corynebacterium pseudotuberculosis*. The exam of the patient on the farm enables to check the ambient conditions in which it was kept, plus the herd as a whole, which helped in drafting the presumptive diagnosis and therapeutic management. In addition, understanding of the epidemiology of the region is an important diagnostic tool in herd medicine. The *C. pseudotuberculosis* has the ability to spread via hematogenous causing abscesses in various organs, however, did not find any report in the literature of brain abscess by that agent. Goat in this report, changes were found in the mediastinal lymph nodes not only, as well as pulmonary abnormalities and pericardial adhesion in lung lobes.

Conclusions: The *Corynebacterium tuberculosis*, the causative agent of lymphadenitis caseous causes the formation of brain abscess. The mechanism is the spread of infection by hematogenous route. The main important way to make the conclusive diagnosis is the necropsy and microbiological culture of the pus.

Small Ruminants

P01-001-118

Assessment of the efficacy, productivity and milk quality response of an Eprinomectin Pour-On when used on milking sheep in Greece.

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Small Ruminants

P01-001-117

Cerebral abscess by *Corynebacterium pseudotuberculosis* in caprine: case report

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Objectives: This multi-centric Greek field study was conducted on 12 sheep dairy farms to demonstrate the efficacy of Eprinex® Pour-On (5g/L Eprinomectin) in lactating sheep and to then determine whether the observed level of nematode control was associated with improvements in Milk Yield (MY) and Somatic Cell Counts (SCC). The unique pharmacokinetic profile of Eprinomectin enables its registered use in lactating cows in many countries. Numerous publications confirm the potential for milk yield benefits both after Eprinomectin usage in dairy cattle and after effective worm control programs being implemented for sheep.

Materials and Methods: Twelve farms were included and 3 trial groups used. For each farm, Group 1 (12 sheep) were not treated whilst sheep in the other two groups (18 in total) received a dose of 1 mg/kg topically of Eprinex on day 0 of the study. Group 2 (12 sheep) received no further treatments whereas group 3 (6 sheep) were treated on two further occasions, 8 weeks apart. Individual fecal samples were collected on day -14 and then weekly from day 0 to day 84 for FEC determination. MY and SCC measurements were routinely collected and were recorded for the study on the same days as the FECs (from day 7). The flocks were seasonally lambing with ewes being included in the study in the early stage of lactation but not necessarily on precisely the same day post-lambing.

Results: All results refer to the average over all the flocks for each group. On day 0, the control FECs ranged from 290-1130 eggs per gram (epg), with an overall average of 676. Groups 2 & 3 ranged from 480-1035 and 325-892 epg with averages of 603 and 595 epg. The majority of nematodes present were identified as *Teladorsagia* spp. At day 84, the control group average was 791 epg with groups 2 and 3 calculated at 226 and 19 epg. Group 2 achieved a low result of 11.1 epg on day 7 (98.2% reduction). The Day 0 MY was not recorded. By day 7, group 2 was producing 50 mL/sheep/day more than the controls, with group 3 sheep producing 34 mL more than group 2. The group weekly production curves were of similar shape over the trial period but the gaps between them widened from day 7, especially between the controls and both of the treated groups. From day 7 to day 84, group 2 produced 10.82L more per sheep than the control group. Group 3 produced 3.26L more per sheep than group 2. The SCC weekly values were recorded on 8 of the 12 farms. On day 7 the average SCC results were 1180, 993 and 911 for groups 1, 2 & 3. The results each week for the control animals trended upwards, reaching 1249 by day 84. Both treated groups trended down and reached 939 and 872 by day 84. Statistical analysis has not been performed on any of these results.

Conclusions: This study confirmed that the topical usage of 1 mg/kg of Eprinomectin in early lactation was effective in reducing FECs. The results also demonstrated a sizeable numerical increase in MY from treatment (10+ L/sheep), with over 75% of the benefit coming from the first treatment, and a noticeable improvement in the SCC in the treated sheep over the study period. These results support the potential for Eprinomectin (Eprinex pour-on) to be an effective anthelmintic for sheep and for its use to be economically beneficial in adult milking sheep. The recommended label dose for cattle is 0.5 mg/kg.

Eprinex is a registered trademark of Merial Limited.

Small Ruminants

P01-001-119

Comparative genomic identification of β -defensin genes in the *Ovis aries* genome

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Objectives: β -defensins are small, cationic, antimicrobial peptides that play a role in immunoregulation across all vertebrates. Though bovine β -defensin genes have been well characterised recently, the genes in *Ovis aries* (domestic sheep) have been poorly annotated, with the majority of genes only identified by automated gene prediction algorithms. The objective of this study was therefore to use a comparative genomics approach to identify and functionally characterise the β -defensin gene repertoire in sheep.

Materials and Methods: In this study, we use all 57 bovine β -defensin genes to find orthologous sequences in the most recent version of the sheep genome. Blast hits with less than 70% identity and 50% coverage were omitted and those with sufficient identity were analysed using BLAST-like alignment tool (BLAT) in order to find their chromosomal location. BioEdit software was used to perform multiple sequence alignments to determine the protein sequence similarity. Putative defensin encoding sequences were then annotated via a phylogenetic analysis using MEGA software.

Results: Of the 57 genes found in the Bovine genome, 25 were found to have close genomic matches, with a large cluster of those genes found on sheep chromosome 13 and smaller cluster found on chromosome 26. Two novel genes were discovered on chromosome 13 and show highest similarity to that of bBD125a and bBD128. As well as novel genes, 10 genes previously annotated as variations of oBD118 and oBD119 have been newly identified as oBD132, oBD142 and oBD117-124. In the chromosome 26 cluster, one gene was found to match fourteen different bovine genes on chromosome 27 with almost uniform similarity. Preliminary comparative analysis of the gene suggests its function may be most similar to that of lingual antimicrobial peptide (LAP).

Conclusions: The identified oBD genes on chromosome 13 illustrate a greater level of defensin gene complexity within the sheep genome than previously thought. The order of these genes correspond directly with bovine chromosome 13, indicating synteny between these two regions. The lack of variety of genes on sheep chromosome 26 in comparison to bovine chromosome 27 suggests a bovine specific expansion, possibly in response to host specific pathogenic threats. This variation may explain the divergent susceptibility of both species to diseases which affect ruminants.

Small Ruminants

P01-001-120

Risks and Managing Copper Deficiency in Sheep and Goats

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Objectives: Copper related disease issues are well-characterized in ruminant species. Sheep are well-known to be sensitive to copper toxicity and often it is limited in the diet. Microbial-induced ruminal interactions between dietary copper (Cu), molybdenum (Mo) and sulfur (S) have been intensively studied and recognized in managing Cu nutrition of ruminants. This is a case study where copper deficiency presented as stillborn and weak lambs and kids and the objective was to define and correct underlying issue to improve animal health and performance.

Materials and Methods: The case study farm raises sheep and goat for meat production. The flock consists of 250 ewes (Finn, Dorset, Suffolk and crossbreds) and 60 Boer does with 200% and 185% lambing and kidding percentage, respectively. Animals are intensively managed and fed a total mixed ration (TMR) throughout the year. All forages are raised locally. Approximately 6 years previous a down Finn ewe was diagnosed with demyelination and nursing kids were diagnosed with enzootic ataxia. Following these initial problems the flock experienced a high rate (17-25%) of stillborn lambs and kids as well as weak neonates with high mortality within 3-4 days of life. Reproductive performance of ewes and does was considered good with 90-95% pregnancy within two service periods. Diagnostic samples of lamb and kid mortalities were submitted to the Animal Diagnostic Laboratory at Pennsylvania State University for a flock workup. Hepatic liver mineral concentrations and vitamins A and E were determined and reported on dry weight (DW) basis. Feed ingredient analyses were completed on a yearly basis. Once diagnosis was made, dietary modification was used to correct the situation and monitored over a 3-year period.

Results: Stillborn kids submitted to the diagnostic laboratory showed no gross abnormalities on necropsy and no positive microbiologic findings. Previous diagnostic investigations showed no infectious causes for the kid and lamb deaths. Hepatic Cu and molybdenum (Mo) in 9 kids was $28.6 \pm 22.2 \mu\text{g/g DW}$ (reference: 75-300 $\mu\text{g/g DW}$) and $3.6 \pm 1.1 \mu\text{g/g DW}$ (reference: 1.5-3.0 $\mu\text{g/g DW}$). Four dead lambs had 36.3 ± 16.7 (reference: 60-300 $\mu\text{g/g DW}$) and $1.9 \pm 0.6 \mu\text{g/g DW}$ hepatic Cu and Mo concentrations, respectively. Similarly, 3 affected ewes had liver Cu and Mo concentrations of 18 ± 13.1 and $3.3 \pm 1.6 \mu\text{g/g DW}$, respectively. Flock diets in 2012 consisted primarily of corn silage and oatlage with additional protein and mineral/vitamin supplements. Forage Cu and Mo content was 6.6 and 5.48 ppm and 7.4 and 5.3 ppm for corn silage and oatlage, respectively. Intent of dietary modification was to increase dietary Cu (37 ppm) supplementation to a level to maintain a 7-8:1 Cu:Mo ratio in the diet resulting in a total dietary Cu concentration of 29 ppm. Lamb and kid losses the following year were reduced in half (6-8% total losses). Forages in 2013 had similar Cu and Mo concentrations and a high Cu diet was fed to pregnant ewes and does with similar positive responses in lamb and kid survival. In 2015 the flock experienced severe (90-95%) lamb losses in all Suffolk or Suffolk cross ewes. In late 2014 a new alfalfa forage was fed to pregnant ewes that was determined to contain 15.4 ppm Cu and 22.1 ppm Mo. Removal of this forage from gestation diets for later lambing ewes reduced lamb losses.

Conclusions: This was an unusual clinical presentation of Cu deficiency as stillbirths and weak neonates are not commonly considered a consequence of Cu deficiency. High concentration of forage Mo (>5.5 ppm DM) relative to Cu (< 12 ppm DM) was responsible for low dietary Cu availability. Recommended dietary Cu:Mo ratio for sheep and goats is 6-8:1 and 6-10:1, respectively. This case study underscores the need to have forages evaluated for Mo and S status in evaluating level of dietary Cu supplementation to ensure adequate availability.

Small Ruminants

P01-001-121

Histophilus somni: an emerging infectious disease pathogen of sheep from Brazil

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Objectives: Histophilosis is disease complex caused by the gram negative bacterium *Histophilus somni* (formerly *Haemophilus somnus*), which involves several clinical syndromes including thrombotic meningoencephalitis (TME), pleuritis, arthritis, bronchopneumonia, septicemia, myocarditis, infertility, and abortion in ruminants. However, the disease occurs predominantly in cattle with sporadic reports in several species of small ruminants. The objectives of this study were to describe the pathological manifestations and molecular finding associated with *H. somni* in sheep from Southern Brazil

Materials and Methods: Sheep (n=9) from this study were from different geographical locations within the state of Paraná, southern Brazil. The clinical manifestations of all animals were observed and a routine necropsy was done soon after death. Selected tissues were fixed by immersion in 10% buffered formalin solution and routinely processed for histopathological evaluation. Duplicate sections of the selected organs (brain, lungs, and heart) were collected freshly during necropsy, and maintained at -80°C until processed for molecular diagnostics. Nucleic acids extracted from tissue fragments of all animals, were used in PCR assays designed to amplify specific amplicons of major bacterial pathogens of ruminants. These assays targeting the 16s rRNA gene of *H. somni*, species and type-specific isolates of *Pasteurella multocida*, and the *lktC-artJ* intergenic region of *Mannheimia haemolytica*. Further, a PCR assay to identify the *rpoB* gene of *Trueperella pyogenes* was done in animal with abscesses. Positive controls and negative controls were included in all PCR assays. PCR products were separated by electrophoresis in 2% agarose gels, stained with ethidium bromide, and examined under ultraviolet light. The amplified PCR products were then purified and submitted for direct sequencing.

Results: Sheep were of different breeds and both genders (8, males; 1, females), and a fetus of one ewe. The principal clinical manifestations were respiratory difficulties (n=7) and apathy (n=3); three ewes had a history of abortion. Significant pathological findings included bronchopneumonia (n=6), necrotizing myocarditis (n=4), thrombotic hepatitis and meningoencephalitis (n=2), pulmonary abscesses (n=2), thrombotic nephritis (n=1), and pulmonary edema and congestion (n=1). *H. somni* DNA was amplified from multiple tissues of all sheep and the fetus. In addition, *T. pyogenes* DNA was amplified from the pulmonary abscesses of one sheep; this animal also contained *H. somni* DNA in multiple tissues. Direct sequencing confirmed the *H. somni* and *T. pyogenes* PCR results. Bacterial DNA was not amplified from the *P. multocida* and *M. haemolytica* PCR assays.

Conclusions: The pathological finding observed during this investigation are consistent with previous descriptions of *H. somni* in ruminants. Confirmation of the participation of *H. somni* in these lesions was confirmed by PCR and sequencing. Further, the amplifying of *T. pyogenes* DNA in a sheep with histophilosis indicates a mixed infection in this animal. Taken together, this study has identified several syndromes of histophilosis (bronchopneumonia, myocarditis, TME, and abortion) in sheep, and support the theory that *H. somni* is an emerging infectious disease pathogen of ruminants from Brazil.

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Small Ruminants

P01-001-122

Effect of Exposure Males to Alternated and Non-Alternated Estrogenized Females and Subsequently Exposed to Anestrous Goats

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Objectives: The effectiveness to induce reproductive response in anestrus mix-breed goats exposed to previously stimulated males throughout exposure to alternated and non-alternated estrogenized females during the natural anestrus season in Northern Mexico (26° N), was tested.

Materials and Methods: The first stage of the study started early in March, where bucks (n=12) with homogeneous body weight were randomly distributed to three experimental groups to be exposed to estrogenized females (n=8; 2 mg estradiol cypionate i.m. every 3d x 10d): 1) ALT (n=4), males exposed to four estrogenized females; two of which were changed every 12 h periods (alternated, novelty effect); males remained alone 1 h between alternations, 2) NALT (n=4), males exposed to two estrogenized goats for a 12 h period; males remained alone 1h, then the same goats were reintroduced with the males for another 12 h period (non-alternated. Non-novelty effect), 3) CONT (n=4), males exposed to two non-estrogenized, non-alternated, control-females, treated with saline every 3 d x 10 d; female management was the same as in group NALT. The second stage of the study started on March 15, where males from the three experimental treatments were exposed to adult anovulatory goats (n=72). Thereafter, goats randomly divided in three experimental groups according to the male treatment groups; ALT (n=24), NALT (n=24) and CONT (n=24). Estrus activity was recorded upon male exposure every 12 h x 14 d. On day 10, the percentage of ovulations across treatments was determined and the day 45 was performed to pregnancy rate. Dates regarding estrus onset, ovulation and pregnancy rates were analyzed by X² while estrus latency (h) through t-student test (SYSTAT 12).

Results: The estrus response during first 10 days was higher (P<0.05) in the ALT and NALT groups, regarding the CONT group (80%, 80% and 0%, respectively). Also, estrus latency (hours) differed (P<0.05) among experimental groups with observed respective values of 89±5.85 h, 106.74±5.40 h and non-estrus response for the ALT, NALT and CONT groups. No differences (P>0.05) were observed regarding the ovulatory response between the ALT and NALT groups (63% and 58%), yet the CONT group got the lowest value (0%; P<0.05). In addition, the ALT and NALT groups had highest pregnancy rates (P<0.05; 58% and 58%) regarding the CONT group (0%). Results demonstrated that, when males were primed with estrogenized females, both the “female effect” and the “female novelty effect” were equally able to induce the “male effect” once bucks were subsequently exposed to anovulatory goats, without major differences regarding of the reproductive outcomes observed in these females. The only observed difference was that regarding to the latency of estrus, favoring to the alternated-females; that is, the “female novelty effect”. The control anestrous females never responded to the males exposed to the non-estrogenized female group.

Conclusions: Results could have important practical implications when designing socio-sexual cues-dependent strategies, clean, green and ethical, in order to induce out-of-season reproductive activity in seasonal breeds, particularly in dairy goat production systems.

Small Ruminants

P01-001-123

INDUCTION OF SEXUAL ACTIVITY IN ANESTROUS DORPER EWES USING FGA or P4 plus eCG THROUGHOUT TWO ROUTES OF ADMINISTRATION

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Objectives: The aim of this study was to evaluate the effectiveness of two different routes, intravaginal (IV) or intramuscular (IM), of progesterone (P4) or fluorogestone acetate (FGA) + equine chorionic gonadotropin (eCG) upon estrus induction and reproductive performance of Dorper anestrous ewes in Northern Mexico (26° N).

Materials and Methods: In February, Dorper adult anestrous ewes (n=48, 3-5 yr. old) with similar body weight (BW) and body condition score (BCS) were subjected to two transrectal ultrasound screenings (HS-2000, Honda Electronics Co., LTD.) on days -14 and -7 prior the onset of the experimental treatments to confirm absence of functional corpora lutea. Thereafter, ewes were randomly distributed to three experimental groups: 1). IVG (n=14; 41.5±3.0 kg BW, 2.0±0.2 BCS), receiving an intravaginal sponge impregnated with 40 mg of FGA, 2). IMG1 (n=17; 41.7±4.0 kg BW, 2.1±0.2 BCS) receiving 20 mg of progesterone i.m., 5 and 2 days prior eCG administration, and 3). IMG2 (n=17; 40.2±3.5 kg BW, 2.0±0.2 BCS) receiving 20 mg of progesterone i.m. 6 and 3 days prior eCG administration. After progesterone treatments, the three groups were administered a dose of 300 IU eCG i.m. (day 0). On day 10, the percentage of ovulations across treatments was determined by detecting the presence of corpora lutea throughout ultrasonographic scanning (USS; HS-2000, Honda Electronics CO, LTD). Then, on day 45, another transrectal-USS was performed to determine pregnancy rate. Estrus activity, ovarian activity and pregnancy were evaluated by comparing thru X² the daily and cumulative proportions of these variables (MYSTAT 12 X2 program).

Results: Our results showed that estrus response during the first 10 days differed (P<0.05) across treatments with the IVG depicting the largest response (100 %) with respect to IMG1 (53 %) and IMG2 (35%). The same was true regarding ovulation percentage with the largest (P<0.05) value observed in the IVG (93 %) followed by the IMG1 (59 %) and IMG2 (53 %). In addition, the highest pregnancy rate differed across experimental groups with the largest value (P<0.05) depicted by the IVG, followed by IMG1 and IMG2 with 24 %.

Conclusions: Results of this study showed that the use of intravaginal sponges impregnated with FGA during a short period of time (6 days) was the best reproductive strategy to induce estrus in Dorper adult ewe during the anestrous season. Such results arise as an interesting option in the out-of-season reproductive management, mainly of highly seasonal dairy ewes breeds.

Small Ruminants

P01-001-124

REPRODUCTIVE PERFORMANCE OF ALPINE ANESTROUS GOATS USING TWO DIFFERENT DOSES OF P4 + eCG UNDER NATURAL MATING OR ARTIFICIAL INSEMINATION

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Objectives: The aim of this study was to evaluate the effectiveness of two different doses of progesterone (P4) plus equine chorionic gonadotropic hormone (eCG) during the anestrus season in goats, exposed either to artificial insemination (AI) and natural mating (NM), in northern Mexico (26° N).

Materials and Methods: Sexually active bucks (n=4) and multiparous anestrus Alpine goats (n=40), were considered. Early in May, goats with homogeneous body condition score (3.0±0.11) were randomly distributed to four experimental groups (10 per group): 1). GAI10, receiving 10 mg P4 i.m., 2). GAI20, receiving 20 mg P4 i.m., 3). GNM10, receiving 10 mg P4 i.m. and 4). GNM20, 20 mg P4 i.m. Upon estrus occurrence, the GAI10 and GAI20 goats were inseminated with frozen semen of known fertility while the GNM10 and GNM20 goats were bred by two sexually active and experienced mix-breed adult bucks, of proven libido and fertility. All female goats received a single dose of 100 IU of eCG, 24 h before of administration of P4 (day 0); the interval to the onset of estrus was registered up to day 15. On day 10, the percentage of ovulations across treatments was determined by detecting the presence of corpora lutea throughout ultrasonographic scanning (USS; HS-2000, Honda Electronics CO, LTD). Then, on day 45, another transrectal-USS was performed to determine pregnancy rate. The estrus response, ovulation and pregnancy rates were analyzed by X2 while estrus latency considered a t-student test (SYSTAT 12, Evenston, ILL, USA).

Results: The estrus response during first 10 days was similar (P>0.05) in all groups (GNM10 90%; GNM20 80%; GAI10 100%; GAI20 90%). Also, estrus latency (hours) did not differ (P>0.05) among experimental groups (GNM10 49.40±3.77; GNM20 55.5±4.2; GAI10 46.80±2.15 and GAI20 46.67±2.28). However, the largest ovulation rate (p<0.05) was observed in the GNM10 (90%) and GAI10 (80%) groups with intermediate values in the GNM20 (70%) and the lowest ovulation rate (P>0.05) depicted by the GAI20 group. In addition, the highest pregnancy rate (P<0.05) occurred in the GNM10 (90%) and GAI10 (80%) groups, with the lowest value in the GAI20 (0%). Analyses of the different doses of progesterone, irrespectively of the AI or NM protocol, generated a pregnancy rate of 55% with the 10 mg P4 vs. 20% obtained with 20 mg P4. The obtained results demonstrate that P4 doses of either 10 or 20 mg i.m. + 100 IU of eCG were equally able to induce estrus response (> 80%) in Alpine goats during the anestrus season.

Conclusions: Yet, those goats treated with 10 mg P4 got increased pregnancy rates regarding those treated with 20 mg P4, irrespectively of insemination protocol. These findings are of practical significance when designing estrus induction protocols in seasonal goat breeds; the last emerges as an important implication, particularly to the dairy goat industry.

Small Ruminants

P01-001-125

The recording of myoelectrical activity of gastrointestinal tract by telemetry electromyography method in sheep

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Objectives: The regulation of gastrointestinal (GI) motility is strongly modulated by different mechanisms. The GI tract contractility is generated by contractions of the myometrial smooth muscle cells that compose most of the forestomachs, abomasum and intestinal wall. The electrical activity of the smooth muscle fibers during contraction or relaxation can be detected as an electromyography (EMG) signal. The aim of the present study was to adapt radiotelemetry technique for EMG recording of the GI as well as evaluated parameters of motility before and during feeding in sheep.

Materials and Methods: The study was carried out using 6 mature crossbred ewes, each weighing 35-40 kg B.W. The animal were kept in metabolic cages throughout the whole experiment. The experiment was started by surgery. Under general anesthesia three silver bipolar electrodes were inserted into muscular layers of the reticulum, the rumen and the abomasum. The combination of electrodes was connected to a three-channel transmitter (TL10M3-D70-EEE, DSI, USA), which was placed under abdominal skin. The EMG signal sent by the implant as radio waves were recorded using the A10/DL10 output- DSI (located approximately 50 cm from cage), coupled to PowerLab and PC computer. Then off-line analysis of EMG signals were performed. Mean amplitude - A [mV], mean RMS (root mean square) [mV], mean duration of electrical activity - D [s] and burst frequency - F [Hz] were analyzed before and during feeding.

Results: The EMG parameters values (mean±SEM) in the abomasum were: A [mV] 0.44±0.01, 0.62±0.01; RMS [mV] 0.14±0.001, 0.19±0.002; D [s] 2.28± 0.04, 2.63±0.04; F [Hz] 0.11, 0.11 before and during feeding respectively. In the rumen were: A [mV] 0.39±0.01, 0.37±0.006; RMS [mV] 0.08±0.001; 0.087±0.001; D [s] 28.62±0.59, 10.92±0.14; F [Hz] 0.02, 0.05 respectively. As well as in the reticulum were: A [mV] 2.30±0.08, 2.01±0.05; RMS [mV] 0.75±0.02, 0.72±0.02; D [s] 13.56±0.27, 12.90±0.21; F [Hz] 0.01, 0.03 respectively. We found that: (1) all measures were obtainable; (2) there were extremely significant differences (P<0.0001) between all measured parameters in each of studied GI regions both before and during feeding; (3) the extremely significant (P<0.0001) increase of D, A, RMS in abomasum and RMS in rumen were observed; (4) the extremely significant (P<0.0001) decrease of D in rumen and significant (P<0.0008) of A in reticulum were observed; (5) there were no differences of A in rumen (P=0.573) as well as D (P=0.052) and RMS (P=0.276) in reticulum between signals recorded before and during feeding respectively; (6) the feeding- depended burst frequency increase in rumen and reticulum were noticed.

Conclusions: It may be concluded that the telemetry electromyography method successfully allowed long term recording of EMG signals from the rumen, reticulum and abomasum in sheep. This reliable method preceded by ordinary surgical procedures allowed to all motility parameters estimation. The preliminary study may provide useful reference values for further investigation.

Small Ruminants

P01-001-127

Comparison of a routine Capillary Electrophoresis method and Single Radial Immunodiffusion assay to measure immunoglobulins in sheep whey colostrumCarlotta Ceniti¹Vincenzo Lopreiato¹Valeria Maria Morittu¹Francesca Trimboli¹Elisabetta Fratto²Genesio Massimini¹Domenico Britti¹¹Università Magna Graecia of Catanzaro, ²Università Magna Graecia of Catanzaro, Catanzaro, Italy

Objectives: In early life, colostrum Igs absorption is critical for the protection of the newborn from infectious diseases. Single Radial Immunodiffusion Assay (SRID) is currently considered the gold standard method for the direct measurements of Igs both in colostrum and serum. Capillary Electrophoresis (CE) is an alternative method capable to directly measure Igs in biological liquids. We had demonstrated that a routine CE instrument can measure Igs in sheep colostrum. The aim of this work was to evaluate the relationship between SRID and CE for the measurement of Igs concentration in sheep colostrum.

Materials and Methods: During the summer of 2014, the colostrum from 12 Sarda sheeps, at 6th-7th lactations, was sampled immediately after lambing (T0) and at 24 hours post partum (T24). The blood samples were collected from the corresponding 12 newborn lambs at 24 hours after birth. Colostrum Igs concentration was determined by a routine CE instrument (Minicap, Sebia France), after fat and caseins removal by centrifugation and enzyme clotting (rennet), respectively. Serum Igs concentration was also determined by CE, as routine. Igs peak in the colostrum electropherogram was identified comparing two electropherograms of a same sample obtained before and after Ig subtraction using Protein G Sepharose conjugate (Gammabind G Sepharose, GE Healthcare). Colostrum and serum IgG concentrations were measured by a commercial SRID kit (IDRing® Box – Sheep & Goat IgG TEST; IDBiotech, France) following the manufacturer's instructions. Before statistical analysis, all colostrum CE data (T0 and T24) and all colostrum SRID data (T0 and T24) were respectively pooled on a same distribution. Data were tested for normality of distribution using the Shapiro-Wilk test (SAS Institute Inc., Cary, NC, USA). For each sample type (colostrum and serum), regression analysis between CE and SRID values was calculated by REG procedure of SAS 9.3 (SAS Institute, Inc., Cary, NC, USA). In addition, for each sample type, Pearson correlation test between the used methods was performed. Statistical significance was declared at $P < 0.05$.

Results: The normality distribution test showed that the considered group of data (CE colostrum, SRID colostrum, CE serum, and SRID serum) were normally distributed. The colostrum Igs concentrations, obtained using CE technique and SRID were, on average, 6.58 ± 4.75 g/dL and 6.42 ± 4.90 g/dL, respectively. The linear regression equation for this set of data was $y=0.40247 + 0.96131x$, $r^2 = 0.98$, showing a strong correlation between the methods ($P < 0.0001$). Linear regression equation ($y=-0.10983 + 0.97458x$) on serum results showed a strong correlation ($r^2 = 0.98$) between the methods ($P < 0.0001$).

Conclusions: We found that, in sheep colostrum, Igs values measured by a routine CE method are linearly correlated with IgG values obtained by a SRID assay. Moreover, because it is known that, differently from SRID, CE analysis measures all together the Igs's classes (IgG, IgA, and IgM) and IgGs represents the major class, our results suggest that in CE analysis the contribution of the minor Ig classes is not significant. Finally, the results of our preliminary study indicate that CE may be a useful tool in the measurements of total Igs in colostrum and could be a rapid and simple approach to assess the colostrum quality.

Small Ruminants

P01-001-128

Epidemiology of endoparasites in small ruminants in Austria, impact of clinical parameters and control of anthelmintic resistanceJulia Schoiswohl^{1,*}¹Department for Farm Animals and Veterinary Public Health, University Clinic for Ruminants, Vienne, Austria

Objectives: The objective of this study was to investigate occurrence of endoparasite infections in small ruminants in Austria and the influence of different Federal States of Austria with different climatic conditions. Additionally, the effect of clinical parameters of endoparasite infestation was evaluated and the control of endoparasites and anthelmintic management were analysed

Materials and Methods: Faecal samples, on the one hand herd samples and on the other hand individual samples from 337 individual animals and 53 small ruminant herds were collected. Individual faecal samples from randomly selected animals were taken and examined for the presence of nematode eggs, trematodes, cestodes and lung worms. In addition all selected small ruminants were clinically examined. Herd faecal samples were also examined for the presence of nematode eggs, trematodes, cestodes and lung worms. All faecal samples (individual and herd samples) were prepared by sedimentation/flotation, sedimentation and the Baermann examination. In addition to the qualitative analyse a quantitative analyse by McMaster counting technique with a detection limit of 50 eggs per gram of faeces (epg) was done. The whole laboratory procedure was performed according to the rules of the Standard Operating Procedure (SOP) of the Institute of Parasitology of the University of Veterinary Medicine, Vienna.

Furthermore, a faecal egg count reduction test was accomplished. Selected animals were randomly assigned to a treatment and control group. All animals were weighted and the small ruminants according to the treatment group were dewormed based on the determined weight. The control group was left untreated.

Results: Nematode infestation was detected in most of examined sheep and goats and also in all investigated herds. Protostrongylides were found in six flocks of sheep and in all flock of goats and the individual prevalence of Protostrongylides varies between about 10% and over 50%. The prevalence of *Dicrocoelium dendriticum* and *Fasciola hepatica* seem to be different between the various regions in Austria.

Correlations between some clinical parameters were detected for sheep and goats.

The effect of the used anthelmintic wasn't satisfactory in all herds and the egg count reduction and also the calculated lower confidence interval show a reduced effect of the used drug.

Conclusions: Endoparasite infestation is common in sheep and goats in Austria and there are variations between different Federal States of Austria with different climatic conditions. Anthelmintic resistance is an increasing problem and a high risk for small ruminants. The reduced efficacy of anthelmintic provides both, a medical and also an economic problem and should not be underestimated. So, it is very important to practice a well-considered and consequential anthelmintic management in cooperation with the farmer to minimize the problem of resistance.

Small Ruminants

P01-001-129

Evaluation of the impact of vaccination against staphylococcal mastitis in milk yield in assaf ewes in Spain.

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Objectives: Staphylococci are the main pathogens responsible for mastitis in dairy sheep flocks. Implementation of a mastitis control program is an essential step in improving milk quality and disease prevention. One of the measures included in these control programs is vaccination. The objective of this study was to evaluate the impact of vaccinating against Staphylococcal mastitis (composed by a Staphylococcus aureus inactivated, expressing Biofilm strain.) on milk yield. The relationship between subclinical mastitis and milk yield is well described in the literature. Mastitis is strongly correlated with a decline in milk yield.

Materials and Methods: The study was performed in an intensively managed dairy sheep flock in the Palencia region (Spain) in which Coagulase-Negative Staphylococci-related subclinical mastitis was present (around 1 million bulk tank somatic cells per mL). Recorded clinical mastitis cases were below 1% per year. One hundred and eight sheep were randomly assigned to one of two groups: i) group V, which consisted of 47 animals that were vaccinated (VIMCO, Laboratorios Hipra, S.A.), according to the recommended administration program; and ii) group C which consisted of 61 animals that were not vaccinated. Management of both groups was the same.

This farm has individual milk yield measurement and total lactation milk yield estimation (Gerionte Oc.) based on monthly records. These data allowed proper monitoring of the daily milk yield (DMY), total lactation milk yield (LMY), and days in milk (DIM). Results were segmented into three categories depending on the lactation number: 1st to 3rd lactation (period A), 4th to 5th (period B) and 6th or more (period C).

Results: The vaccinated animals (group V) produced more milk (18.6%) in fewer days (12.9) than the non-vaccinated group (group C). In group V, results of DMY, LMY and DIM were 1.255 L, 273.38 L and 221 days, respectively, in period A, 1.166 L, 227.54 L and 190 days, respectively, in period B, and 0.946 L, 164.19 L and 169 days, respectively, in period C. In group C, values of DMY, LMY and DIM were: 1.182 L, 239.43 L and 210 days, respectively, in period A, 1.077 L, 176.85 L and 201 days, respectively, in period B, and 0.967 L, 195.29 L and 189 days, respectively, in period C. In the overall data DMY and LMY were higher (7.84% and 16.82, respectively) with an average DIM 12.9 days shorter. Animals with more than five lactations did not show any improvement with the vaccination which might be due to a chronic infection and lesions in the udder.

Conclusions: Subclinical Staphylococcal mastitis prevention through vaccination (VIMCO) can contribute to increase milk yield in sheep. In this case, there was a difference of milk production of 35 L per animal which may have a direct economic benefit of €30 (calculated using a milk price of €0.85/L) based on milk production alone.

Small Ruminants

P01-001-130

First case of tick-borne encephalitis in sheep in Germany

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Objectives: Tick-borne encephalitis (TBE) is the most important viral tick borne zoonosis in Europe. In Germany, the highest incidence is reported from the federal states Bavaria and Baden-Württemberg with about 250 annual human cases. TBE is caused by the TBE virus (TBEV, Flaviviridae) which is transmitted by ticks, particularly Ixodes ricinus or alimentary by unpasteurized milk or milk products from viramic ruminants. In veterinary medicine only sporadic cases in dogs, horses, small ruminants and a monkey have been reported despite a high number of wild and domestic animals tested TBEV seropositive.

Materials and Methods: In May 2015, a five month old lamb from a farm with 15 Merino Land Sheeps and followers showed an impaired general health with pyrexia and acute neurological signs. The sheep suffered from a torticollis, tremor, nystagmus, salivation and finally somnolence with inappetence and immobility. The veterinarian suspected listeriosis. The animal was euthanized and submitted for pathological examination. Following necropsy, a histopathological, immunohistochemical, bacteriological, parasitological and virological analysis was performed. Additionally, sera of all sheeps and followers were examined serologically.

Results: At necropsy, the sheep showed a moderate to severe infestation with Trichostrongylus species, Eimeria species and Moniezia species. Histopathology revealed a mild to moderate, necrotizing, lymphohistiocytic and granulocytic meningoencephalitis with gliosis and neuronophagia. Immunohistochemistry for TBEV was negative. RNA of TBEV was detected in the brain by real-time RT-PCR and subsequent PCR product sequencing. A phylogenetic analysis revealed a close relationship to "Tick-borne encephalitis virus strain Kumlinge A52". TBEV could be cultured from brain tissue. Serologically, two of 21 blood samples were questionable positive for TEBV in an ELISA and one sample was positive in a serum-neutralization test.

Conclusions: To the authors knowledge this is the first report of a naturally TEBV infection in a sheep in Germany with clinical manifestation. TBEV infection should be considered as a differential diagnosis in a sheep with neurological signs.

Small Ruminants

P01-001-131

Phimosis occurrence in lambs in a feedlot and their association to breeds

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Objectives: The aim was to assess the presence of phimosis, congenital decreased of the preputial orifice diameter that impedes the protrusion of the penis, in a population of lambs in a feedlot and correlate with different breeds of sheep.

Materials and Methods: were used 200 sheep, from five different breeds or crosses (Dorper, Ile de France, Santa Inês, Suffolk and Texel) aged between two and three months old, with average weight of 31.04 ± 3.20 kg from a feedlot of the Teaching Veterinary Hospital of the FMV – Aracatuba campus of the Univ Estadual Paulista “Julio de Mesquita Filho”- Unesp. For evaluation, to verify the presence or not of phimosis, the animals were contained seated and performed the exhibition of the penis. Data were noted in tables (presence or absence of phimosis) and then statistically analyzed using the GraphPad Stat program v 3.10. Fisher’s exact test was used to verify the association between presence/absence of phimosis and breed. The presence of phimosis was dependent on the breed of the evaluated animals ($p < 0.0005$).

Results: From the 200 evaluated sheep, 48 (24.00%) had phimosis and five (2.50%) had urethral obstruction. The presence of phimosis is related to the Ile de France breed because of the 36 animals of this breed, 16 had phimosis (44.44%). Of the 38 Santa Inês animals evaluated, seven (18.42%) had phimosis. Among the 74 animals of the Suffolk breed analyzed, 22 (29.73%) had phimosis. Of the 37 Dorper animals evaluated, three (8.11%) had phimosis. None of the 15 animals of Texel assessed presented phimosis.

Conclusions: We conclude that the Ile de France breed is more predisposed to phimosis and that the high incidence of phimosis observed in feedlot, draws attention to the importance of evaluating the penis, especially in those destined for reproduction, since the presence of this condition impairs reproductive function.

Small Ruminants

P01-001-132

Pulmonary cryptococcoma with encephalitic dissemination in a goat from Midwestern Brazil

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Objectives: Cryptococcosis is a mycotic disease of humans and domestic animals that is caused by the *Cryptococcus neoformans* complex of organisms, includes into two distinct species: *C. neoformans* and *C. gattii*. Most descriptions of cryptococcosis in domestic animals have occurred in dogs and cats, with few cases in other species. The objective of this study was to describe the pathological and immunohistochemical findings associated with pulmonary cryptococcosis with encephalitic dissemination in a goat.

Materials and Methods: A 3-year-old, mixed breed, female goat with clinical manifestations of progressive behavioural changes and disequilibrium was admitted at the Veterinary Teaching Hospital, Universidade de Cuiabá, Midwestern Brazil. This goat was maintained on a small farm that contained 16 goats reared on a semi-intensive system, where pigeons are frequently observed and ingest part of the

feed provided to goats. A samples of the cerebrospinal fluid (CSF) was collected for cytological evaluation. The clinical manifestations progressed and the animal died spontaneously despite adequate therapy. A necropsy was done soon after death and selected tissues were routinely processed for routine histopathological evaluation with the Hematoxylin and Eosin (H&E) method. Duplicate sections of the brain were stained by the mucicarmine histochemical method, and for the immunohistochemical (IHC) typing of the cryptococcal species by using monoclonal antibodies (mAb471, mAb302, and mAbF10F5) to stain the cryptococcal capsule. In addition, formalin fixed paraffin embedded (FFPE) tissue fragments of the brain were used in a fungal PCR designed to amplify the Internal Transcribed Spacers (ITS) regions I and II.

Results: Cytological evaluation of the centrifuged CSF revealed fungal organisms with morphology consistent with *Cryptococcus* sp. Grossly, a large space occupying gelatinous mass was observed at the left caudal pulmonary lobe, and several similar smaller masses at the frontal and temporal cortex, the thalamus, hippocampus and mesencephalon. The H&E evaluation of the pulmonary mass revealed large quantities of fine-budding, negatively stained thick-walled fungal organisms loose within the lung or within the cytoplasm of macrophages and a discrete influx of inflammatory cells; a distinct granulomatous reaction was not observed. Similar histopathological findings were observed within the intracerebral masses. The mucicarmine stain clearly imparted a pinkish coloration to the fungal organisms and the negatively stained thick-wall observed by H&E within the masses of the lung and the brain. Additionally, the mAb471 antibody positively labelled the capsule of all organisms within the *Cryptococcus neoformans* complex; the mAb302 antibody imparted a positive immunoreaction to the capsule of *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans* organisms; while the mAbF10F5 antibody positively labelled the capsules of *C. neoformans* var. *grubii* and *C. gattii* organisms. The interpretation of these IHC findings indicated that the intralesional thick-walled, fine-budding mycotic organisms observed by the H&E and the mucicarmine stains correspond to *C. neoformans* var. *grubii*. However, fungal DNA was not amplified from the FFPE tissues sections during the ITS PCR assays.

Conclusions: The fungal organisms observed by cytological and histopathological within the lungs and several areas of the brain are consistent with *Cryptococcus* spp., while the gelatinous masses in these organs are typical of cryptococcal granulomas (cryptococcomas). Further, the mucicarmine stain identified the intralesional fungal organisms as *Cryptococcus neoformans*, while IHC typing characterized the organisms as *C. neoformans* var. *grubii*, being diagnostic for cryptococcal organisms. Although the exact method of infection is unknown, it’s rather likely that the disease first affected the lungs with subsequent encephalitic dissemination.

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P01-001-133

Virulence factors of coagulase-negative Staphylococci isolated from sheep with subclinical mastitis and their relationships with cure rates

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Objectives: The objectives of the present study were to identify the species of Coagulase-negative Staphylococci in milk of mastitic ewes with and without antimicrobial treatment, and to investigate the presence of genes relating to resistance of β -lactam antimicrobials, formation of biofilms, production of enterotoxins and production of the toxic shock syndrome toxin.

Materials and Methods: Sixty sheep were divided into three groups: G1, without treatment; G2, animals treated via the intramammary route with 100 mg of cloxacillin during drying off; and G3, sheep treated via the intramammary route with 50 mg of nanoparticulate cloxacillin. Milk samples were gathered during drying off and 15 and 30 days after the parturition of the subsequent lactation. The analyses to identify the species of Coagulase-negative Staphylococci were carried out by means of the internal transcribe spacer technique and the investigation of the genes responsible for the virulence factors and resistance to oxacillin was performed using the polymerase chain reaction (PCR) technique. To determine the relationship between the variables was carried out the multiple correspondence analysis.

Results: *Staphylococcus warneri* was the most prevalent microorganism. No sample was positive for the *mecA* gene. The only gene relating to production of enterotoxins was *sec*. Among the genes relating to production of biofilm, *icaD* was the only one identified in the three experimental groups. Six untreated sheep present *S. warneri* with virulence factor genes before weaning. All of them continued to present subclinical mastitis during the subsequent lactation. Two untreated sheep with *S. epidermidis* isolation before weaning did not present spontaneous recovery 15 and 30 days after parturition. One of these presented microorganisms with the *icaD* gene, while the other concomitantly presented the genes *icaC* and *icaD*. In G2, mammary halves infected by *S. simulans*, which presented the *icaD* gene, and by *S. epidermidis*, which carried the *sec* gene, did not attain a cure after treatment for subclinical mastitis. Three sheep presented reinfection 30 days after parturition, after being treated with 100 mg of cloxacillin-benzathine (G2). Out of these, one presented *S. warneri* carrying the gene *icaD*, another presented *S. xylosum* carrying the gene *bap* and the third presented *S. chromogenes* with no occurrence of virulence factors during the pretreatment period. In G3, the cases of lack of cure by 15 and 30 days after parturition occurred in mammary halves infected by *S. epidermidis*, *S. warneri* and *S. simulans*. The gene *icaC* was observed during the pretreatment period among sheep infected by *S. epidermidis*, while the gene *icaD* was observed among animals infected by *S. warneri* and *S. simulans*.

Conclusions: The defense mechanisms of the mammary gland and the treatment for subclinical mastitis during the drying off period did not eliminate all species of CNS after parturition. Coagulase-negative Staphylococci that carries genes responsible for production of enterotoxins and biofilms can negatively interfere in the cure rates for mastitis among sheep.

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P01-001-134

Footrot Characterization in Serra Da Estrela Native Flocks

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Objectives: Serra da Estrela native (SEN) breed of sheep is one of the most important in Portugal, standing responsible for the production of one of the most internationally recognized cheeses in the world, “Queijo Serra da Estrela” (protected designation of origin). One of the major risks to the sustainability of SEN dairy flocks is footrot, an infectious disease that causes lameness, decrease in milk production, weight loss and decrease in fertility. The aim of this work was to determine which parameters become decisive for the appearance of footrot in SEN dairy flocks, while establishing associations with environmental and nutritional variables.

Materials and Methods: The present study was carried out by performing an especially designed 27 multiple choice questionnaire, based on the underlying causes of lameness in livestock and in the clinical diagnosis performed by the vet technician of the clinical cases evaluated at the moment of the inquiry. The survey was performed during the execution of the official veterinary health program between February and September of 2014 by a veterinarian team from the Association of SEN Sheep Breeders (ANCOSE). The ovine producers (N=30, with a total of 1270 animals) were randomly selected from the extensive area of production of “Queijo Serra da Estrela”. The parameters evaluated in the study were: season and consequent weather changes during the period of the study, floor types, hygiene conditions, bed types, the existence and use of footbaths, location of footbaths, foot trimming and foot hygiene procedures. After the construction of the database and using the Statistical Package for Social Sciences version 16.0 the frequency response for each item was calculated.

Results: All SEN livestock producers are proactive in the treatment of lameness (70%). About 99.7% of lameness was related with footrot and most appeared in winter (36.7%). In some occasions there was not a specific season distribution (56.7% rainiest years). From the analyzed farms, 70% use straw as a floor bed, followed by bush (21%). 45.6% of animals were clinically diagnosed with footrot and were sheltered in straw floor. Bed quality is good in 40%, however frequent changes in the floor bed in the preceding 15 days had a higher rate of footrot diagnosed cases (33,23%) compared to monthly changes. Regarding sheepfold animal number, the distribution is proper in 36.7%, elevated in 30%, overcrowded in 6.7% and low in 23.3%. Clinically ill diagnosed animals in last category were the lowest observed (3%). Concerning hoof trimming, 76.7% trims while 23.3% reported not to perform that task. From those that perform trimming, 73.9% do it only when necessary, 21.7% at the time of clipping and 4.4% when the animal is affected. One curious result was that animals who perform trimming at clipping had higher footrot cases (52.6%), unlike those who trim only when necessary (40.2%) or even when animals are clinically ill (0.91%). Mostly all in the presence of footrot choose a local treatment (95.2%) using antibiotic sprays, instead of parenteral antibiotic treatment. Footbath is rarely used in the prevention (13.3%) of this disease and when it is misconceived (25%) and incorrectly formulated (100%).

Conclusions: This study was the first performed in Portugal focusing footrot characterization in native SEN flocks. Economic impact in SEN dairy flocks is attributed to factors such as direct decrease in milk and meat production, early slaughter of affected animals due to non responsive treatment and medical expenses. The most effective eradication method combines first of all the ability to understand the problems of SEN producers and implementation of preventive measures and treatment of footrot. Often linked to lack of formation, the advance age of portuguese producers the mentality and social factors block the advance of veterinarian intervention itself.

Small Ruminants

P01-001-135

Heat tolerance environmental indices for Brazilian hair sheep

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Objectives: The aim of the present study was to analyze if four commonly used heat tolerance and seven environmental indices are appropriate to evaluate heat stress in Santa Ines and Morada Nova sheep and if the limits used to classify thermal stress are suitable for these breeds.

Materials and Methods: The experiment was repeated six times at the beginning of winter period in Brasilia, Brazil. Twenty-six sheep from two genetic groups were used and data were collected twice a day, with a total of 156 observations.

Thermographic photos and physiological parameters such as respiratory rate, heart rate and rectal temperature were collected in the morning and afternoon.

Physical variables included: shoulder height, thoracic perimeter, back and body lengths, rump height, rump width, breast width, skin thickness, number of hairs, hair length, skin and coat color.

The following heat tolerance test based on animal measures were calculated: Iberia, Benezra test, Rauschenbach-Yerokhin (RY) and Baccari.

The environment was characterized using the following measurements: Black globe temperature (TBG), dry bulb temperature (TDB), relative humidity (RH), wet bulb temperature (TWB) and dew point (Td). Based on these data, the following indices were calculated: Temperature and Humidity Indices (THI): $THI1 = (0.55 \times TDB + 0.2 \times Td) \times 1.8 + 32 + 17.5$; $THI2 = [0.4 \times (TDB + TWB)] \times 1.8 + 32 + 15$; $THI3 = (0.8 \times TDB) + [(RH/100) \times (TDB - 14.4)] + 46.4$; $THI4 = TDB + (0.36 \times TDB) + 41.2$; $THI5 = (1.8 \times TDB + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times TDB - 26.8)]$; $THI6 = (TDB + TWB) \times 0.72 + 40.6$; Black Globe-Humidity Index (BGHI) = $TBG + 0.36Td + 41.5$;

Statistical analysis was performed using SAS version 9.3 (Statistical Analysis Institute, Cary, North Carolina). The procedures used included correlations and "Broken-Line" regressions.

Results: High daily air temperature variation (14.6 - 43.2°C) was seen during the experiment with high average temperature and low relative humidity (13%) in the afternoon. High environmental indices (THI2: 85.31) were also observed in the afternoon, reaching values classified as danger (THI: 79-83) and emergency (THI ≥84).

Correlations between environmental and physiological parameters were positive, with higher correlation between rectal temperature and THI3 (0.71). Regarding heat tolerance indices, rectal temperature was negatively correlated with Iberia index (-0.44) and positively with the others. The Benezra coefficient was the only one to correlate significantly with all the physiological parameters, showing perfect correlation with respiratory rate (1.00). A negative correlation was observed between the Iberia index and the morphometric measures and positive correlation between these measures and rectal temperature, Benezra, Bacarri and RY heat tolerance indices.

In general, correlations between surface temperature and environmental indices were high and positive, with higher correlations between the temperature of the neck and THI3 and THI5 (0.89).

Regarding heat tolerance indices, the Benezra was the best correlated with surface temperatures with correlation of 0.52 between this index and foot temperature.

The environmental indices were best related with the temperature of the neck and foot. The inflexion points between the rectal temperatures and environmental indices varied according to the equation used, with higher values for the THI1 (95.55) and THI2 (81.32).

Conclusions: The high correlation between environmental indices and rectal and skin temperatures demonstrates that these indices are good tools to evaluate thermal comfort of sheep. However, in order to classify these appropriately the equation used needs to be carefully studied, because these classifying values can vary according to the species and equation. Regarding the heat tolerance tests, Iberia and Benezra indices were best correlated with the parameters studied.

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P01-001-136

Heat tolerance in Brazilian Santa Ines and Morada Nova hair sheep

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Objectives: The aim of the present research was to evaluate heat tolerance using heat tolerance environmental indices, physiological, physical, and hematological parameters in Santa Ines and Morada Nova sheep in Brazil.

Materials and Methods: The experiment was repeated six times at the beginning of winter period in Brasilia, Brazil. Twenty-six sheep from two genetic groups (Morada Nova (MN) and Santa Ines (SI)) were used and data were collected twice a day, with a total of 156 observations.

Physiological and hematological parameters such as respiratory rate (RR), heart rate (HR), rectal temperature (RT), number of erythrocytes (RBC), leukocytes (WBC), platelets (PLAT), concentration of hemoglobin (HB), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) Packed Cell Volume (PCV), total plasma proteins (TPP), plasma fibrinogen (FIBR) and thermographic photos were collected on three days in both morning and afternoon.

Physical variables included: shoulder height (SH), thoracic perimeter (TP), back (BKL) and body (BL) lengths, rump height (RH), rump width (RW), breast width (BW), skin thickness (ST), number of hairs (NH), hair length (HL), skin and coat color.

The following heat tolerance tests based on animal measures were calculated: Iberia and Benezra. The environment was characterized using the following measurements: dry bulb temperature (TDB) and relative humidity (RH). Based on these data, the following indices were calculated: $THI = (0.8 \times TDB) + [(RH/100) \times (TDB - 14.4)] + 46.4$; Statistical analysis was performed using SAS version 9.3. The procedures used included analysis of variance, correlation and principal component analysis. Means were compared using adjust Tukey test with a significance level of 5% ($p < 0.05$).

Results: High daily air temperature variation (14.6 - 43.2°C) was seen

during the experiment with high average temperature and low relative humidity (13%) in the afternoon. THI reached values classified as danger (THI: 79-83) and emergency (THI ≥84) in the afternoon.

The RT, RR, thermograph temperatures and the Benezra index were higher during the afternoon and most blood parameters were lower in this period. Santa Ines animals were larger and had longer, greater number and darker hair, thicker skin, higher RR, TPP, HB, MCHC, WBC, Benezra index and lower Iberia index.

RT was negatively correlated with skin brightness. Regarding heat tolerance indices, size measurements, skin and hair brightness were negatively correlated with Benezra. Surface temperatures were positively correlated with RT, RR and Benezra index, with highest correlations with RT. Regarding blood parameters, a direct relationship was noted between heart rate, number of red blood cells and hemoglobin concentration and negative correlation between these blood parameters and Iberia index and THI.

The principal component analysis showed that 47% of the variation was explained by physiological and physical variables of the animals. High rectal temperature, larger animals and thicker skin were related with higher Benezra and lower Iberia index. Regarding surface temperatures, 56% of the variation was explained by the characteristics studied. It was noted a direct relation between these temperatures, physiological components, THI and Benezra index and indirect with Iberia index.

Conclusions: The environmental index indicated that sheep suffered heat stress during the afternoon, but due to the good adaptation of MN and SI breeds, these animals were able to maintain their physiological and blood parameters within reference values. Although both breeds can be considered adapted to the environmental conditions, MN breed may be more suitable for farming in the Midwest region because of its physical characteristics which favors heat tolerance.

Small Ruminants

P01-001-137

Electroencephalography for assessing pain and analgesia in sheep

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Objectives: Numerous painful husbandry procedures are performed routinely within the Australian sheep industry. The assessment of analgesia is limited by current pain detection methods such as behaviour and observation. The use of electroencephalography (EEG) in relation to therapeutic efficacy is used in human medicine (pharmacology-EEG), and offers potential as an effective and practical way of measuring pain response and efficacy of analgesia in livestock. The aim of this study was to evaluate the electroencephalographic response of conscious sheep with and without the administration of the non-analgesic sedative midazolam.

Materials and Methods: The EEG responses for two ewes and two wethers (n=4) were recorded prior to and post administration of midazolam for a minimum of 5 minutes. Raw data was band-passed filtered offline between 0.1-40Hz and visually inspected with traces containing significant movement artifacts discarded. The maximum power frequency (f_{PM}) was calculated using LabChart Version 7 software (AD Instruments) and analysed using restricted maximal likelihood regression (REML) for linear mixed models analysis in Genstat (V16). Effect of sheep and time (pre and post administration of midazolam) were assessed in the model.

Results: There was a significant effect of sheep on f_{PM} ($P < 0.001$). The two ewes (Sheep 1 and 2) had higher mean f_{PM} (22.5 ± 0.013 and 6.95 ± 0.008 Hz respectively) than the two wethers (Sheep 3 and 4, 0.773 ± 0.015 and 0.764 ± 0.015 Hz respectively). This suggests there may be an effect of sex on f_{PM} , further analysis using larger sample sizes is required. There was a significant effect of time on f_{PM} ($p < 0.001$). There was a significant reduction in f_{PM} following administration of midazolam in all sheep. Mean f_{PM} was $9.052 (\pm 0.009)$ Hz before and $6.439 (\pm 0.01)$ Hz after midazolam administration. These results indicate a reduction in movement artifact following non-analgesic sedation, however further work is needed to compare the effects of pain and analgesia on f_{PM} .

Conclusions: Results indicate a significant effect of sedation on the maximum power frequency of the EEG output. A decrease in mean f_{PM} suggests a reduction in movement artifact, allowing for more accurate EEG analysis of perceived pain in conscious sheep. Effects of sex need to be further explored using larger sample sizes. The use of non-analgesic sedation will allow for animals to remain conscious, reducing costs and eliminating impracticality elements of complete sedation. Use of pharmacologyEEG methods in sheep will allow for practical on-farm assessment for analgesic interventions for painful husbandry procedures.

Small Ruminants

P01-001-138

Amilorating Effects Of Cysteine, Glutathione And Taurine During Freezability Of Ram Spermatozoa

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Objectives: Ram sperm have a higher ratio of polyunsaturated/saturated fatty acids and a lower ratio of cholesterol/phospholipids molar than other species, which renders the sperm much more vulnerable to oxidative damage caused by reactive oxygen species (ROS). The aim of the current study was to evaluate the effects of cysteine, glutathione and taurine on the freezability of ram spermatozoa and biochemical parameters

Materials and Methods: Semen samples from ten mature mixed Barki rams were used in this study. Pooled semen samples were divided randomly into 7 aliquots one was diluted with a Shotor extender as a control basal diluent while others in addition to basal extender L-Cysteine, L- reduced glutathione and taurine were added by two levels for each to present seven experimental groups. Diluted semen cooled to 5°C then frozen by liquid nitrogen in 0.25 ml French straws. After 48 hr frozen, straws were then thawed individually at 37 °C for 30 sec. in a water bath then subjected to evaluation.

Results: The obtained results showed that, addition of glutathione by a rate of 5mM significantly increases motility than control group. Similarly, addition of taurine by a rate of 50mM significantly increased the viability index of cryopreserved semen samples.

Percentages of acrosomal defects were dramatically decreased ($P < 0.05$) with glutathione (10 mM) addition compared to control (9.00 ± 1.16 % and 21.00 ± 0.58 % respectively).

In regards to sperm membrane integrity, cysteine (5 mM) provided a greater protective effect (47.00 ± 1.16) compared to control (% 35.33 ± 0.88 %).

Antioxidant addition showed decreased enzyme leakage and significantly ($P < 0.05$) improved total antioxidant capacity (TAC) especially taurine (50Mm) compared to control group.

In regards to DNA integrity, taurine (50mM) provided a significant protective effect (98.51 ± 0.42 %) compared to control.

Conclusions: Findings of this study showed that addition of cysteine, glutathione and taurine to semen extenders, of greater benefit to quality of frozen-thawed ram sperm. However further investigation on their effect on fertilizing capacity and pregnancy rate still needed.

Small Ruminants

P01-001-140

Sexual behaviour, semen quality and testosterone levels in Saint Croix hair sheep breed rams under extensive pasture scheme in a semi-desert region

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Objectives: the objective of this research was to evaluate semen quality, sexual behavior traits and testosterone levels during the year and the influence of feed supplementation in Saint Croix breed adult rams under semi-desert climate conditions in Northeast Mexico

Materials and Methods: This research was conducted at the Laboratory for Animal Reproduction of Marín Academic Unit, Faculty of Agriculture, Autonomous University of Nuevo Leon, which is located in Marín, NL, Mexico. Its geographic coordinates are $25^{\circ} 54'$ north latitude and $99^{\circ} 58'$ west longitude, at an altitude of 451 meters. Temperatures range from $18-43^{\circ}\text{C}$ in summer and 10 to -2°C in the winter time. The average annual temperature is 23.1°C with an average annual rainfall of 429 mm (INIFAP, 2009). Sixteen adult rams were used, randomly divided into grazing group (GG) and supplemented group (SG). Each group consisted of 8 rams that grazed on buffel grass pasture; rams from SG were additionally supplemented with a concentrate of 14 % crude protein and 2.5 Mcal of metabolizable energy (1kg/animal/day). Every two weeks, semen was collected by artificial vagina, sexual behavior traits were evaluated and sera were taken to determine testosterone levels. The statistical analysis of variance was made using SPSS (SPSS, version 19, 2008) statistical package with linear model, where supplementation and month of evaluation were used as independent variables. When significant difference was found, the comparison of means by the minimal significant difference was performed.

Results: In general, SG males had better body condition score and showed higher live weight gain ($P < 0.05$) than the GG rams. However, regarding scrotal circumference, statistical difference was found only in the month of evaluation ($P < 0.05$). For semen quality traits, statistical

difference was observed ($P < 0.05$) in the month of evaluation with respect to the SG. The values of some semen quality traits were higher or equal in the GG than in the SG, especially during the rainy periods (from April to May and from August to October). Concerning the sexual behavior traits, statistical difference of feed supplementation and month of the year was found ($P < 0.05$) on reaction time, sniffing and flehmen trait values, with the highest values recorded in the SG from March to May. Furthermore, a statistical difference was found ($P < 0.05$) in both the month of evaluation and supplementation on every feature of the sexual behavior.

Regarding testosterone levels, the SG had statistically significant interaction in the month of evaluation ($P < 0.05$), with the highest values being recorded in June (summer time) and October (autumn term) and the GG had the lowest in spring season (March-May) compared to the SG.

Conclusions: It is concluded that rams of Saint Croix breeds are able to adapt to adverse environmental and nutrition conditions in semi-desert climates and can be used to induce heat and mate the ewes in any reproductive season. However, with good mating and nutrition management, improved sexual behavior, semen quality parameters, and testosterone levels are achieved which improves the reproductive efficiency of the herds extensively raised in those regions at global level.

Comments: It is a work that took place in northeastern Mexico in a hair sheep breed adapted to semi-desert regions.

Small Ruminants

P01-001-141

Teat-End Hyperkeratosis In Dairy Ewes And Its Predisposing Factors

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Objectives: Although teat-end hyperkeratosis is a condition well known and described in dairy cows, relative information is scarce in dairy ewes. The objective of the present study was to assess teat-end hyperkeratosis in dairy ewes raised under Mediterranean conditions.

Materials and Methods: A random sample of 28 dairy sheep flocks was used. The average flock comprised 229 ± 146.0 milking ewes with an average milk yield of $ca 273 \pm 71.7$ kg/lactation period. All flocks were visited by the same veterinarians that assessed milking procedure using a designated recording sheet. Records included use of gloves by milkers, pre-stripping, clusters removal after vacuum cessation and post-milking disinfection. Technical characteristics of the milking parlour and relative equipment were also assessed in terms of their functionality and general condition. A designated equipment (Exendis Milking System Analyser, PTV) was used to measure vacuum level at teat-end, pulsation rate and pulsation ratio in 3 randomly selected milking units of the milking parlour. The vacuum level in the vacuum pump was also recorded; the difference of vacuum level between clusters and vacuum pump was calculated. In each flock, a random sample of 20% of the milking ewes was subjected to a detailed teat-end examination for the assessment of hyperkeratosis using the 4-scale evaluation method described by Mein et al. (2001). Evaluation was performed immediately after cluster removal. The problem was considered serious when scores 3+4 and/

or 4 were above 20% and 10%, respectively. Data were analysed using univariate analysis of variance with SPSS 22, statistical software. Vacuum level, difference of vacuum level between clusters and vacuum pump, pulsation rate, pulsation ratio and clusters removal after vacuum cessation were transformed into categorical variables and used as fixed effects.

Results: Average prevalence (%) of hyperkeratosis score 1, 2, 3 and 4, at teat-level, was 69.3, 15.5, 9.9 and 5.2, respectively. The analyses at flock level showed that 39% of the flocks (11/28) had a serious problem of teat-end hyperkeratosis. In five out of the 11 flocks scores 3+4 were assigned to 30.2% of teat-ends; from them in 14.5% of cases the hyperkeratosis score was equal to 4. The other 5 flocks had scores 3+4 assigned to 23.9%, whereas in one flock, score 4 was assigned to 12.5% of teat-ends. The effect of vacuum level on teat-end hyperkeratosis was significant ($P < 0.05$). In flocks where the vacuum level of the milking parlour was >40 kPa a significantly ($P < 0.05$) higher percentage of teat-ends scored 3+4 was found. The effects of other factors assessed were not significant ($P > 0.05$).

Conclusions: A high prevalence of teat-end hyperkeratosis in dairy ewes was observed; nearly 40% of the examined flocks had a serious problem. In most cases, the higher percentage of teat-ends scored 3+4 was observed in flocks milked with higher vacuum level. The latter suggested that teat-end hyperkeratosis in dairy ewes may be closely related to the functionality and the general conditions of the milking parlour.

Comments: References: Mein, G.A., Neijenhuis, F., Morgan, W.F., Reinemann, D.J., Hillerton, J.E., Baines, J.R., Ohnstad, I., Rasmussen, M.D., Timms, L., Britt, J.S., Farnsworth, R., Cook N., and Hemling, T. 2001. Proceedings of the 2nd International Symposium on Mastitis and Milk Quality.

Small Ruminants

P01-001-142

Clinical cases in sheep and goat attended by the GEPECO from Universidade Federal Fluminense at Rio de Janeiro State, Brazil

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Objectives: The main goal is to report clinical cases in sheep and goats attended by the Group of Study, Research and Extension in Goats and Sheep (GEPECO) from Faculty of Veterinary at Universidade Federal Fluminense in Rio de Janeiro State in partnership with animal diagnostic laboratories of the Instituto Biológico in Sao Paulo.

Materials and Methods: A retrospective study was carried out from October 2012 to October 2015 at Rio de Janeiro State. A total of eight farms of meat sheep, seven of dairy goats, two of meat goats and one of dairy sheep were encompassed. Clinical data were collected from the field routine of the GEPECO with local sheep and goat farmers. For microbiological and histopathological diagnosis of some illness, biological material was collected from sick or necropsied animals and sent to laboratories of virology, bacteriology and pathology of the

Instituto Biológico in Sao Paulo.

Results: A total of 733 clinical cases were attended and separated by: infectious(333/733), parasitic(206/733), reproductive(113/733), metabolic (34/733) and traumatic cases (47/733). Infectious cases: contagious ecthyma (54/333), dermatophilosis (48/333), caseous lymphadenitis (47/333), keratoconjunctivitis (42/333), scours (30/333) pneumonia (29/333), bluetongue (24/333), mastitis (21/333), enterotoxemia (9/333), caprine arthritis encephalitis (14/333), papillomatosis (8/333), foot rot (5/333) and tetanus (2/333). Parasitic diseases: hemoncosis (53/206), eimeriosis (41/206), monieziosis (38/206), cutaneous myiasis (33/206), oesophagostomosis (25/206), visceral cysticercosis (7/206), pediculosis (6/206), babesiosis and anaplasmosis (3/206). Reproductive findings: hydrometra (53/113), abortions (10/113), cystic endometrial hyperplasia (9/113), follicular cyst (9/113), pyometra (5/113), macerated fetus (4/113), birth defects (4/113), mucometra (3/113), luteal cyst (2/113), retained placenta (3/113), mummified fetus (2/113), uterine prolapse (2/113), polycystic ovary (1/113), cervicitis (1/113), monorchidia (2/113), testicular hypoplasia (1/113), testicular degeneration (1/113) and anorchidia (1/113). Metabolic cases: photosensitization (12/36), seasonal dermatitis (11/36), pregnancy toxemia (9/36) and abomasal ulcers (3/36). Traumatic cases: dog bite (11/47), corneal ulcers (10/47), locomotor system injuries (8/47), abdominal hernia (6/47), ear hematoma (3/47), fracture of long (3/47), cervical (2/47) and sacral bone (1/47), laceration of ear (2/47) and udder (1/47).

Conclusions: The animal health management in small ruminants, compared to cattle, is still scarce by handlers, farmers and field veterinarians. It is highlights the importance of the continuous promotion of health education for people involved with the sheep and goat care in Rio de Janeiro State.

Comments: The authors believe the data contained in this abstract are relevant because its expose to the international scientific community about the diseases found in small ruminants in the southeastern Brazil. Furthermore, these findings may assist in the discussion of the local sanitary control measures.

Small Ruminants

P01-001-143

Case of study: Evaluation of the impact of mastitis vaccination on mastitis treatment in a dairy goat farm in the south-west of Spain

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Objectives: Staphylococci are the main pathogens responsible for mastitis in dairy goat herds. Implementation of a mastitis control program is an essential step in improving milk quality and preventing infection. One of the measures that can be included in these control programs is vaccination. The objective of this field trial was to evaluate the efficacy of vaccination for Staphylococcal mastitis in reducing mastitis treatments in a commercial dairy goat farm.

Materials and Methods: The study was performed in a dairy goat farm (Murciano-Granadina) in the southwest Spain where goats were vaccinated with a commercially available Staphylococcal mastitis vaccine (Vimco, Laboratorios Hipra S.A.). The farm has 550 goats and 5 kidding periods (February, April, June, September and November).

The main mastitis pathogen described is *Staphylococcus* (CNS and *S. aureus*). The vaccination program started in August 2014 and was implemented according to the recommended administration schedule. The study compared mastitis treatments before (monthly average of 2 years) and after (1 year) the first immunization of the herd. No changes other than vaccination occurred during this time. Two different mastitis treatments, which were recorded monthly, were used: (A) antibiotic and nonsteroidal anti-inflammatory drugs (NSAIDs) for severe mastitis and (B) nonsteroidal anti-inflammatory drugs for mild mastitis. Total treatment (A+B) was also calculated.

Results: The number of mastitis treatments per year before vaccination was 88 in group A, 50 in group B, and 138 in the A+B groups combined. After vaccination the number of treatments recorded was 61 in Group A, 5 in Group B and 65 in Groups A+B. Overall, fewer mastitis treatments (A, B and A+B) were required after starting vaccination than before (-31%, -90% and -52%, respectively). The total reduction in treatments (A+B) from before to after vaccination program was introduced as follows: September (-25%), October (-44%), November (-92%), December (-40%), January (-24%), February (-85%), March (-33%), April (-20%), May (5%), June (-11%), July (-68%), and August (-88%). Reduction of mastitis treatments was evident every month, with the exception of May where 10 animals were treated before starting vaccination program in the herd and 11 were treated after.

Conclusions: Results show that vaccination against *Staphylococcal* mastitis can effectively minimize the amount of mastitis treatments (antibiotics and NSAIDs) in the herd. Furthermore, the drop in mastitis treatments was associated with a reduction in mastitis cases. These results suggest that including vaccination in a mastitis control program may be a good approach to prevent the disease and reduce the use of mastitis treatments, thereby improving milk quality and public health.

Small Ruminants

P01-001-144

Reproductive Tract Surgeries in Sheep under General Inhalational Anaesthesia

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Objectives: Sheep (*Ovis aries*) is gaining acceptance as an established model for research about small ruminant reproductive tract physiology. The aim of the study was to record myoelectric activity of the reproductive tract following induced oestrus with different synchronisation protocols during reproductive season.

Materials and Methods: The 9 Polish Landrace sheep were enrolled into the study. The elective surgical procedure under general inhalational anaesthesia was performed in all animals. The general inhalational anaesthesia (sevoflurane in oxygen; Sevoflurane Baxter) with endotracheal intubation following intramuscular premedication (midanium 0,4 mg/kg; Midanium 5mg/ml Polfa Warszawa S.A., ketamine 4mg/kg; Bioketan 50 ml Vetoquinol Biowet PL) and intravenous induction (propofol 4mg/kg; Scanofol 10mg/ml ScanVet Poland) was performed due to the laparotomy. The silicone base silver bipolar electrodes were adapted to be sutured on oviduct and uterine horn. Sheep were surgically fitted

with TL10M3-D70-EEE (DSI, St. Paul, MN, USA) implants positioned between the abdominal muscles, and 3 silicone electrodes sutured on the left or right oviduct (bulb and isthmus) and the corresponding uterus horn. The myoelectric activity was recorded using the DL10 analog output (DSI) coupled to PowerLab (ADInstruments, Melbourne, Australia) and PC computer. The postoperative antibiotic therapy with amoxicillin 1ml/10kg (Betamox L.A 150mg/ml; ScanVet Poland) in combination with postoperative analgesia meloxicam 0,5 mg/kg (Metacam 5mg/ml; Boehringer Ingelheim Vetmedica) was proceed for 5 days. The sheep after the research underwent euthanasia with intravenous injection of phentobarbital 0,3- 0,6 ml/kg (Morbital; Biowet Puławy) to collect tissues for histopathology examination.

Results: The maintenance of general anaesthesia (sevoflurane in oxygen) was smooth and easier to control compared with general anaesthesia maintenance with isoflurane in oxygen. No complications like heart arrhythmias, respiratory depression and anaphylactic reaction were observed during surgery. The recovery process after endotracheal tube removal was much faster (5 min- standing position) without complication in intensive care room for large animals compared with recovery process after isoflurane administration (15 min). The back to consciousness after sevoflurane anaesthesia is faster than after isoflurane anaesthesia which is preferably especially in ruminants.

Conclusions: General inhalational anaesthesia (sevoflurane in oxygen) allows for optimal however safely, painless management of the highly complicated, long surgeries on reproductive tract in sheep. Furthermore, the medical sciences state of art progress affords application of the latest anaesthetic drugs and techniques allowing to minimize pain and distress in animals especially during long surgical procedures.

Small Ruminants

P01-001-145

Left Flank Ovariohysterectomy as The Final Surgical Treatment of the Pseudopregnancy in Pet Goat

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Objectives: Nowadays goats are not only farm animals but also kept as outdoor pets at modern eco type or agro tourism farms in particular. However, individual approach is essential while the treatment routine of the most common pathologies. Pseudopregnancy is one of the major causes of anestrus in goats during the reproductive season. It is characterized by the persistence of a corpus luteum in the absence of a (viable) conceptus in the uterus. The primary clinical feature is hydrometra (accumulation of fluid in the uterus) imitating the normal pregnancy development.

Materials and Methods: The two and a half year old dairy goat was brought to the clinic with severe bilateral abdominal enlargement. The goat had never been naturally mated or artificially inseminated. A clinical examination was done and all the basic life parameters evaluated were normal. The large volume of fluid accumulated inside the uterus was confirmed by ultrasound examination. During the previous as well as the present breeding season, the goat was administered a single dose

of prostaglandin F2 alpha and oxytocin; nevertheless the hydrometra reoccurred. Radical surgical treatment was demanded by the goat owner. A general inhalational anaesthesia (sevoflurane in oxygen; Sevoflurane Baxter) with endotracheal intubation following intramuscular premedication (midanium 0,4 mg/kg, ketamine 4mg/kg) and intravenous induction (propofol 4mg/kg) was chosen in order to perform left flank ovariohysterectomy. During surgery the total amount of accumulated fluid of 15 liters was evacuated prior to removing uterus and ovaries. A postoperative antibiotics penicillin with dihydrostreptomycin 1ml/20 kg (Depomycin; ScanVet Poland) in combination with non-steroidal anti-inflammatory drug flunixin 2 mg/kg (Flunimeg; ScanVet Poland) was administrated.

Results: The maintenance of general anaesthesia (sevoflurane in oxygen) was smooth and easier to control compared with general anaesthesia maintenance with isoflurane in oxygen. No complications like heart arrhythmias, respiratory depression and anaphylactic reaction were observed during surgery. The recovery process after endotracheal tube removal was much faster (5 min- standing position) without complication in intensive care room for large animals compared with recovery process after isoflurane administration (15 min). The back to consciousness after sevoflurane anaesthesia is faster than after isoflurane anaesthesia which is preferably especially in ruminants.

Conclusions: Nowadays goats are not only farm animals but also kept as outdoor pets at modern eco type or agro tourism farms in particular. However, an individual approach is essential when treating the most common pathologies. General inhalational anaesthesia (sevoflurane in oxygen) allows for optimal however safely, painless management of the highly complicated, surgeries on reproductive tract in goats. Furthermore, the medical sciences state of art progress affords application of the latest anaesthetic drugs and techniques allowing to minimize anaesthetic risk, pain and distress of the goat.

Small Ruminants

P01-001-146

General Inhalational Anaesthesia for the Surgery of the Urinary Tract in Goat Model

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Objectives: Goat (*Capra hircus*) is gaining acceptance as an established model for biomedical research and for surgical training moreover in preclinical toxicological testing of pharmaceuticals. Goats can be gentle, easy to handle and transport, intelligent, affectionate, friendly, and clean and are increasingly being used as an alternative to the dog or monkey. The aim of the study was the transurethral injection of the mesenchymal stem cells then urodynamic, follow up examinations at certain intervals.

Materials and Methods:The 30 polish white landrace goats were enrolled into the study. The elective, surgical, research procedure under general inhalational anaesthesia was performed in all animals. The general inhalational anaesthesia (isoflurane in oxygen; Aerrane Baxter) with endotracheal intubation following intramuscular premedication (midanium 0,4 mg/kg; Midanium 5mg/ml Polfa Warszawa S.A. , ketamine 4mg/kg; Bioketan 50 ml Vetoquinol Biowet PL) and intravenous induction

(propofol 4mg/kg; Scanofol 10mg/ml ScanVet Poland) was applied due to the surgery. The postoperative antibiotic therapy with amoxicillin 1ml/10kg (Betamox L.A 150mg/ml; ScanVet Poland) in combination with postoperative analgesia meloxicam 0,5 mg/kg (Metacam 5mg/ml; Boehringer Ingelheim Vetmedica) was proceed for 5 days. Goats after the research underwent euthanasia with intravenous injection of phentobarbital 0,3- 0,6 ml/kg (Morbital; Biowet Pulawy) to collect tissues for histopathology examination and to obtain urethral explants after stem cells transplantation for analysis with In Vivo Imaging System (IVIS).

Results: The maintenance of general anaesthesia (sevoflurane in oxygen) was smooth and easier to control compared with general anaesthesia maintenance with isoflurane in oxygen. No complications like heart arrhythmias, respiratory depression and anaphylactic reaction were observed during surgery. The recovery process after endotracheal tube removal was much faster (5 min- standing position) without complication in intensive care room for large animals compared with recovery process after isoflurane administration (15 min). The back to consciousness after sevoflurane anaesthesia is faster than after isoflurane anaesthesia which is preferably especially in ruminants.

Conclusions: General inhalational anaesthesia allows for optimal however safely, painless management of the highly complicated, research surgeries on reproductive tract in goat model. Furthermore, the medical sciences state of art progress affords application of the research animals however pain and suffer have to be reduced.

Small Ruminants

P01-001-147

Positive effects of Butafosfan with vit B12 (Coforta B12) on the reproductive efficiency in ewes on pasture under a synchronization program in Chile

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Objectives: Butaphosfan with Vit 12 (Coforta B12) is widely used in bovine as a metabolic enhancer, to improve energy balance and metabolic status. There is no information documented about the use of this product to improve the body condition in ewes on pasture with a consequent effect in reproduction parameters. The objective of this paper was to evaluate the reproduction parameters in ewes on pasture and under a synchronization program with progesterone implant

Materials and Methods: The study was done in a farm in the South of Chile. 466 ewes on pasture and under synchronization program with progesterone intravaginal device + prostaglandin F2 alfa. Animals were randomly allocated in 3 groups. The group 1 (232 ewes) remained as control, they received the progesterone device. Groups 2 and 3 (234 animals divided in 2 groups) received after the implantation of the device 5 ml of Coforta B12 (500 mg butaphosfan + 0.25 mg of Vit B12). Group 3 received a second injection (same dose) of Coforta B12 10 days later. After this all groups were taken to the males for breeding (1 male / 25 synchronized ewes). Fertility rate and prolificity (number of lambs/ ewe) were statistically evaluated.

Results: Fertility rate was improved by 7.1% in the group which received one injection of Coforta B12 compared with the control (73.9% vs 66.8%). The group with 2 injections the fertility rate was significantly increased

uo to 87.7% ($p < 0.05$). Better prolificity was detected in the treated groups, in the group 1 was lower (1.13), group 2 showed a rate of 1.22 and 1.32 in the group 3. There was no statistical difference between control group and group 1, but group 3 shows a significant difference in comparison with control group (Kruskal-Wallis statistical method)

Conclusions: Based on the current findings a significant positive influence is detected in those synchronized animals with 2 treatments with Coforta B12, mostly in the fertility rate. In the case of prolificity the numerical difference shows also a significant benefit

Comments: This study could be the basis for future ones, considering animals on pasture, with potential problems in body condition that can affect the reproduction parameters.

Small Ruminants

P01-001-148

Effect of phosphorus and calcium supplementation on goat performance communally grazed in a semi-arid area of Republic of South Africa.

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Objectives: The main aim of the research was to evaluate the levels of phosphorus and calcium in goat faeces, blood and in the pasture in the semi-arid area of the North West Province and to assess the effect of selected mineral (P and Ca) supplementation on goat's health and production.

Materials and Methods: In this study, a total number of 24 female goats, two years of age were purchased from local farmers around Mafikeng. The goats were randomly grouped into two groups of 12, the control and the supplemented groups respectively. The supplemented group was fed dicalcium phosphate ad lib in the evenings for 12 months. Faecal, blood and grass samples were collected monthly, in addition to body mass of individual animals. Calcium (Ca), Phosphorus (P) and Magnesium (Mg) samples were analysed using the Spectrophotometer and ICP-MS.

Results: Results obtained showed that Ca and P supplements did not influence both faecal and serum P levels as compared to the untreated goats. However, Ca and P supplemented goats displayed significant ($P < 0.05$) serum Ca and Mg concentrations increase. This showed the significance of supplementing Ca and P supplements to grazing communal goats in this region. In addition, significant ($P < 0.05$) body mass gain were recorded in goats supplemented with Ca and P as compared to non-supplemented goats throughout the trial period, again emphasizing regular P and Ca supplements to grazing goats in semi-arid areas.

Conclusions: The results obtained in this study have confirmed the hypothesis in semi arid areas of south Africa such as Mafikeng, supplementation of Ca and P is needed in semi-arid areas in order to improve productivity in goat farming.

Small Ruminants

P01-001-149

The influence of the chosen various factors on the fecundity of the Olkuska breed during the natural reproductive season

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Objectives: The Olkuska ewes are known to display usually a high litter size. However due to various, sometimes unknown reason this feature is not always clearly demonstrated. The aim of this study was to evaluate the possible methods of increasing the litter size in Olkuska ewes during the natural reproductive season.

Materials and Methods: During experiment 40 adult ewes of Olkuska sheep were used. The animals were randomly divided on three groups. The females from group A ($n=10$) were synchronized with the use of sponges containing 20 mg of flugestone acetate (Chronogest, Intervet) with injection of 450 IU of serum gonadotropin (Folligon, Intervet) at the time of sponges withdrawal. Ewes from group B ($n=10$) were treated according the same protocol with additional administration of Sildenafil citrate as a vaginal suppositories twice with the interval of one day. Suppositories containing 100 mg of active substance were applied deep into vagina after sponges withdrawal and then after next 12 hours. The animals from group C ($n=20$) did not received any protocol and were treated as a control group. Two rams (1 and 2) were used for mating. Every mating was recorded.

Results: Obtained results revealed the highest fertility in the control group (80%, 90% and 95% in group A, B and C respectively) however the highest fecundity were observed in the group B (189%) vs. 150% and 179% in groups A and C respectively. Average number of lambs per ewe was the same in groups B and C (1,7), but in group A was much lower (1,2). Average period from putting rams to lambing was similar in all groups: 150,25 vs. 150, 67 vs. 156,79 days in group A, B and C respectively. Average litter size after both rams mating was also alike: 1,60 and 1,55 in ram 1 and 2 respectively.

Conclusions: Previous study of different authors proved the effectiveness also in sheep and cows of sildenafil as effective and selective inhibitor of phosphodiesterase type 5 (PDE5) in improving the blood flow in reproductive organs (uterus, placenta). This effect achieved by relaxation of the smooth muscle cells of blood vessels what in turn induce of vasodilatation could be responsible for the highest input of the active substances into the reproductive organ and in turn creation of the better environment for the effective ovulation, nidation and pregnancy maintenance.

Comments: However this is just a hypothesis, obtained results suggest the need of further studies performed on the bigger number of animals, which could verify presented hypothesis.

Small Ruminants

P01-001-150

Serum Proteins Of Sheeps Infected With Haemonchus contortus

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Objectives: Population growth associated with the need for less costly agricultural activities makes the sheep industry a profitable alternative to animal protein production. Among the diseases that affect sheep, parasitism by gastrointestinal nematodes, especially *Haemonchus contortus*, represents a serious health problem causing significant economic losses. Acute phase proteins are produced by liver and their synthesis is associated with acute infections and inflammations and are important in the early diseases diagnosis. Therefore, we studied serum protein profile of pure and crossbred sheep experimentally infected by *H. contortus*.

Materials and Methods: 10 lambs, 5 Santa Inês (SI) and 5 crossbred Dorper x Santa Inês (DSI), male, weanling, confined for 60 days at Centro de Pesquisas em Sanidade Animal (CPPAR) - UNESP Jaboticabal. The animals received orally inoculum containing 1,000 infective larvae (L3) of *H. contortus* and were subjected to blood collection in tube without anticoagulant (10 mL) for determination of protein profile by SDS-PAGE technique, and feces collection to perform the EPG (eggs per gram) in basal times (D0), 20, 40 and 60 days after experimental infection (D20, D40 and D60 respectively). For statistics was used completely randomized design submitted to analysis of variance and the means were compared by Tukey test at 5% significance level. Acute phase proteins assayed were: albumin, IgA, IgG, ceruloplasmin, haptoglobin, transferrin and α-acid glycoprotein.

Results: Serum albumin concentrations, ceruloplasmin and transferrin were significantly different between the DSI and SI groups in all studied moments, and had lower albumin concentration in the DSI group when compared to the SI group. For ceruloplasmin and transferrin were observed significantly higher averages in the DSI. IgA concentrations were significantly different only in D20, being the concentration two times lower in SI group when compared to the DSI, however during the entire experimental period DSI group showed higher numerical values of IgA. With regard to IgG, at all times, there was a higher serum concentration in the SI group compared to DSI but with no significant difference. The concentration of proteins haptoglobulin and α- acid glycoprotein showed no significant difference between groups. And the quantity of eggs in feces of SI group was three times higher (1745 epg) than DSI (502 epg) in D40, day with higher egg counts.

Conclusions: This study shows that Dorper X Santa Inês crossbred sheep have a better immune response and consequently more resistance to parasites when compared to Santa Inês sheep, making it a good breeding option when considering susceptibility to parasites.

Objectives: This paper reports a case of penile hematoma in an adult sheep, not due to trauma by sexual activity. This condition is one of the most prevalent disease in the penis, usually occurring during copulation, having been described in cattle but not for sheep, according to the literature.

Materials and Methods: A ram of 18 months of age in reproductive rest and secluded to females, confronted another male after escape. The animal was subjected to systemic non-steroidal anti-inflammatory therapy for three days due to increased volume at the ventral region of perineum and held in sexual rest for 60 days. After five months of trauma, the animal was with inability to expose the penis, although libido, and due a volume of 15cm diameter distal to the perineum, sensitive to palpation and with floating regions. In ultrasonography exam was visualized a structure circumscribed with not homogeneous echogenicity content, suggestive of penile hematoma, confirmed after centesis. Surgery was indicated because of the size of the lesion and time of evolution. The patient underwent intravenous general anesthesia and epidural lumbosacral anesthesia and surgery was initiated with skin incision in the central region of the volume increase. After removal of the clots, it was possible to expose the penis which was surrounded by a fibrous capsule and sigmoid flexure and identify in the site of injury. The fibrous capsule and adjacent penile adhesions were removed, making it impossible to rebuild the tunica albuginea because of their degree of commitment. After the surgery was instituted seven days of penicillin procain, 10 days of flunixin meglumine, and hydrotherapy in testicular scrotal skyn followed by cold compresses on the operated area, three times daily for 10 days. The points of skin were removed after 15d and the patient was discharged, under the supervision of sexual rest for 60 days.

Results: Two months later to the surgery, the patient returned with increased floating testicular volume, without sensibility and with leucopenia and increased fibrinogen. In ultrasonographic examination, an isolated structure involving an irregular echogenic liquid, suggestive of abscess was visualized, confirmed by centesis, being sent to orchiectomy. In addition to this report describe the penile hematoma in sheep, describes a pathogen also not yet been described in the literature as penile injury was due to an indirect trauma rather than direct trauma to the erectile penis during copulation. The location of the volume increase observed in sheep this report, dorsal to the scrotum, also differs from the literature and has been attributed to how the injury occurred without exposure or penile erection, which may have favored a more dorsal rupture the flexure sigmoid.

Conclusions: Complications like abscesses and adherences are common in the penile hematoma, especially in chronic and serious injuries, as was the case in the ram this report. The penile hematoma can occur not only in copulation, but indirect penile trauma with location of injury caudal to the scrotum in sheep. The size of the penile hematoma is an important parameter for the decision of conservative or surgical therapy and precocity in treatment is fundamental to a more favorable prognosis.

Small Ruminants

P01-001-151

Penile hematoma in a Dorper sheep breed: case report

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Small Ruminants

P01-001-152

Estimulation Of Sexual Behavior Of Adult Dorper Rams Treated With Glutamate And(Or) Testosterone During Increased Photoperiods-Spring In Northern Mexico

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Objectives: This study aimed to evaluate of different treatments upon reproductive parameters as well as their effectiveness to induce sexual behavior of rams under natural lighting conditions of spring -increased photoperiods-, the natural sexual resting season in Northern Mexico (25° N, 103° W).

Materials and Methods: Dorper rams (n=20; 2-3 yr. old, 81±0.32 kg body weight (BW); 3.8±0.05 body condition score (BCS) were divided in four homogeneous groups in terms of BW, BCS, scrotal circumference (SC) and odor (OD), and were randomly assigned to four experimental groups: 1). Glutamate Group (GG, n=5; treated 7 mg kg⁻¹ BW of L-Glutamate i.m. every 4d x 30d); 2). Testosterone Group (TG, n=5; treated with 0.5 ml saline every 4d x 15d + 25 mg of testosterone i.m. every 3d x 15d); 3). Glutamate + Testosterone Group, (GTG, n=5; receiving 7 mg kg⁻¹ BW of L-Glutamate every 4d x 30d + 25 mg of testosterone i.m. every 3d x 15d, the last 15d) and 4). Control Group (CONT, n=5; which received 0.5 mL of saline i.m. every 4d x 30d). On days 1, 15 and 30 BW, BCS, SC and OD of rams were measured; OD was classified by smelling the base of the horns at a distance of 15 cm and using a 0-3 scale. After 30d of treatments, two males were randomly selected from each treated group and exposed to four groups of anovulatory ewes (n=14 each; 42±2.36 kg BW; 3.04±0.22 BCS) to evaluate the male sexual behavior, considering three responses: 1). Appetitive sexual behavior (ASB; flehmen, anogenital sniffing, approaches, kicking, vocalizations, draw the penis), 2). Consummatory sexual behavior (CSB; mount attempts and mounts) and 3). Isolation behavior (ISL; standing, escape attempt, lying down and lying down time), during 2-h daily x 2 d. While BW, BCS, SC and OD data were evaluated by ANOVA, sexual behaviors considered X² (SYSTAT 12.0).

Results: No differences (P>0.05) regarding BW, BCS, SC and OD score were observed at the onset of the experiment among experimental groups. The same was true regarding BW, BCS and SC at the end of the experimental period, with 80.9±0.33 kg, 3.5±0.07 units and 35.23±0.2 cm, respectively. Yet, on d15, the GTG depicted the strongest odor score (1.5±0.22, P<0.05) regarding GG, TG and CG (0.75±0.20, 0.4±0.24 and 0.10±0.10, respectively; P>0.05). However, on d30, no differences (P>0.05) occurred among the GTG, GG and TG groups (1.5±0.2, 0.9±0.2 and 0.8±0.3, respectively) with the lowest value detected in the CG (0.6±0.1) which differed with the GTG (P<0.05). Results of sexual behavior showed that in the ASB, the GTG depicted the highest percentage of performed behaviors regarding GG, TG and CONT (43 vs 29, 11 and 18% respectively), yet, only differences (P<0.05) occurred between TG and CONT. In the CSB-phase, the GG and GTG depicted the largest percentage of behaviors, with the lowest values (P<0.05) depicted by both the TG and CONT (20%). In the case of ISL, the CONT accumulated the highest percentage (62%; P<0.05) regarding GTG, GG and GT (25, 13 and 0%, respectively).

Conclusions: Results demonstrate that Dorper rams treated with glutamate + testosterone during the natural sexual resting season in Northern Mexico promoted not only an increased odor score but also an augmented consummatory sexual behavior when exposed to increased photoperiods during the natural resting season in Northern Mexico. Such promissory results encourage delineation of reproductive management strategies to increase reproductive outputs during the out-of-season period in the sheep industry.

Small Ruminants

P01-001-154

Radiographic Lung Alterations In Premature And Full-Term Lambs

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Objectives: The aim of this study was to describe and compare the thoracic radiographic changes in full-term lambs borned by normal delivery and cesarean section and premature lambs delivered by cesarean section. Forty three lambs divided into six groups: two groups were normal for gestational age and four groups preterm.

Materials and Methods: The normal groups were: normal delivery group (NORM, n= 9) and normal for gestational age but delivered by cesarean section group (143 days) (NORMCS, n=7). All the four premature groups were delivered by cesarean section in 138 days of gestation. The premature groups were: premature group (PRE, n=5), premature group whose mothers received dexamethasone (16mg/ IM) 36 hours before surgery (DEX, n=7), premature group that the lambs received surfactant intra tracheal instillation (100mg/kg) immediately after birth (SURF, n=6) and the last one premature group whose mothers received dexamethasone (16mg/ IM) 36 hours before surgery and the lambs received surfactant intra tracheal instillation (100mg/kg) immediately after birth (DEXSURF, n=6). The lateral thoracic radiographs were taken immediately after birth (M0), twenty-four hours (M₂₄) and forty-eight hours (M₄₈) after birth using a portable x-ray machine. All radiographs were made at 40kVp and 2 mA at a 100 cm focus-film distance. Fisher's exact test was used to determine if there were any differences between the groups in each moment; significance was set at P < 0,05. All changes were grouped to perform the statistical analysis, so we have lamb with or without radiographic changes.

Results: Radiographic changes are dependent of the group at M0 (p=0,0337) when compared NORM group with NORMCS group. When compared four premature groups (PRE, DEX, SURF and DEXSURF) the radiographic changes are dependent of the group at M48 (p=0,0057). When compared five groups of lambs delivered by cesarean section (NORMCS, PRE, DEX, SURF and DEXSURF) was not found differences between the moments. Immediately after birth (M0) the radiographs of the SURF group revealed 100% (6/6) of pleural effusion. Radiographic changes were noted in NORM 44.44% (4/9), NORMCS 100% (7/7), PRE 83.3% (5/6), DEX 66.7% (6/9) and DEXSURF 100% (6/6) groups. At M24, 55.6% (5/9) of NORM, 50% (3/6) of NORMCS, 75% (3/4) of PRE, 57.1% (4/7) of DEX, and 100% (5/5) of DEXSURF showed changes. After 48 hours (M48), 77.8% (7/9) of NORM and 66.66% (4/6) of NORMCS showed no changes, 75% (3/4) of PRE lamb showed changes in alveolar patterns and 85.7% (6/7) of DEX lambs showed changes in alveolar patterns (4/6), bronchial patterns (1/6), and interstitial, alveolar and bronchial patterns (1/6). The DEXSURF lambs (100%; 5/5) showed changes in interstitial and alveolar patterns (3/5), and interstitial, alveolar, and bronchial patterns (2/5). All deaths occurred before 24 hours of life. Two lambs died in each of the PRE and DEX groups, six (100%) died in the SURF group, and one died in the NORMCS and DEXSURF treatment groups.

Conclusions: We conclude that if the lambs show diffuse infiltrates, the ones that received a surfactant treatment, will have a poor prognosis. The lambs borned by cesarean section in normal age gestation presented more alterations than the ones spontaneous delivered. The period of 48 hours for lambs borned by normal delivery and normal for gestational

age but delivered by cesarean section was sufficient to remove the lung fluid, while the premature lambs still showed changes in the thoracic radiographic patterns.

Small Ruminants

P01-001-155

A proposal for a classification of phimosis in lambs

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Objectives: The aim is to propose a classification for different degrees of phimosis in lambs, given the lack of information and varied forms of this presentation in these animals, as well as the possible correlation of predisposition to urolithiasis with this anatomical condition.

Materials and Methods: Were evaluated 119 lambs between three and four months old, of different breeds or crosses (Dorper, Ile de France, Santa Inês, Suffolk e Texel), average weight of 31.4 ± 4.68 kg, from a feedlot in the Teaching Veterinary Hospital of the FMV – Aracatuba campus of the Univ Estadual Paulista “Julio de Mesquita Filho”- Unesp. For evaluation, the animals were contained seated, with subsequent penile exposure, verifying the presence or absence of phimosis and a classification in grade, according Kikiros, Beasley and Woodward (1993), adapted to the anatomy of sheep. The following grades were determined: I - normal animals, with full retraction without narrowing behind the glans, with total exposure of the appendix; II - full retraction with narrowing behind the glans; III - partial exposure of the glans and appendix limited by foreskin; IV - discrete retraction, but there is no exposure of the glans, appendix and urethral meatus; V - no foreskin retraction is possible.

Results: According to the proposed classification, normal animals accounted 83.19%. Animals that showed complete retraction with narrowing behind the glans, classified as grade II, amounted to 7.56% (nine animals). The five lambs identified as grade III, accounted for 4.20%. Meanwhile, sheep with a slight retraction, non exposed glans, appendix and urethral meatus were four, totaling 3.36%. Only one of the evaluated animals (0.84%) did not presented any possible foreskin retraction, being classified as grade V.

Conclusions: The classification adapted from Kikiros, Beasley and Woodward (1993) proved to be effective on the identification of degrees of phimosis in lambs. Thus, the proposed method can be applied as a routine practice in the sheep medicine.

Small Ruminants

P01-001-156

Physiological and blood parameters of sheep in Brazil

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Objectives: Reference values used in tropical and subtropical regions are based on sheep raised in temperate climate and those in Brazil are subject to different environmental stressors. Therefore, the aim of the present work was to define reference values for hematological and physiological parameters for sheep in Brazil.

Materials and Methods: A database with 5,081 observations of sheep in nonstressful environmental conditions (THI equal or below 70) with information on time of day, sex, age, breed, physiological and hematological characteristics and environmental variables was elaborated. Because of the variety of breeds, they were grouped into types: commercial wool (CW), locally adapted (LA) and locally adapted x wool (NW).

The physiological and blood characteristics evaluated included: rectal temperature (RT), respiratory rate (RR), heart rate (HR), packed cell volume (PCV), total plasma protein (PPT), number of red blood cells (RBC), hemoglobin concentration (HB), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), number of white blood cells (WBC), monocytes (MONO), lymphocytes (LYMP), eosinophils (EOSI), segmented neutrophils (SEGM), platelets (PLAT) and fibrinogen (FIBR).

Statistical analysis was performed using SAS® (Statistical Analysis Institute, Cary, North Carolina) with significance level of 5%. The procedures used included analysis of variance, averages and confidence interval of 95% (pctpts command in PROC UNIVARIATE).

Results: Most of the means of the studied parameters were within published reference values for the species. However, the values of 2.5 and 97.5 percentiles, which indicate the lower and upper limits, respectively, differed from those previously established in textbooks. Respiratory rate was the parameter showing highest divergence, with values far above normal range (20-34 movements/minute) for sheep in all types and age groups. Similarly, upper and lower heart rate limits were not within reference intervals (70-80 movements/minute), and in CW adult group the average heart rate (113.51 beats/minute) was also higher than values previously reported. The maximum rectal temperatures of CW (40.7°C) and LA (40.3°C) adult sheep and animals young were also higher than those considered as normal (38,3 - 39,9°C).

The lower and upper limits of packed cell volume were lower than the reference values (27-45%), and the 2.5 percentile was outside the normality range. In general, minimum values of the number of red blood cells and hemoglobin concentration of all types and age groups also differed from reference limits.

Mean number of leukocytes was within the reference range, however maximum and minimum limits were different. A greater number of red blood cells, lower concentration of hemoglobin and MCHC were observed in young sheep. Adult animals had higher TPP, PLAT and FIBR. As for the type, CW had higher TPP (7.51g/dl) and LA adult higher PLAT (734.36 x 10³/µL). In the leucogram, lower number of leukocytes was observed in young NW group (7.47 x 10⁶/µL).

Conclusions: Although means of most parameters were within textbook reference ranges for sheep, the upper and lower limits of many variables were different from the reference values, with significant number of observations outside reference interval. Therefore, considering that physiological and hematological parameters are essential to evaluate pathological disorders, animal welfare and adaptability to the environment, the use of reference values based on animals with similar breed characteristics and subjected to similar environmental conditions is recommended for a correct interpretation of the results.

Small Ruminants

P01-001-157

Serum Ceruloplasmin, Haptoglobin And A1-Acid Glycoprotein As Markers Of Induced Mastitis In GoatsKalina Simplicio¹, Diana C. C. Sanchez¹, Hugo B. Brunetti¹, Thaís G. Rocha¹, Ana Maria C. V. Martins², José J. Fagliari³¹Veterinary Clinics Department, São Paulo State University, UNESP, Brazil, Jaboticabal, SP, ²São Paulo University, USP, Pirassununga, ³Veterinary Clinics Department, São Paulo State University, UNESP, Jaboticabal, SP, Brazil

Objectives: Acute phase proteins (APP) are sensitive and early indicators of inflammation. However, information regarding goats APP are scarce. Therefore the aim of this study was to determine serum concentration of ceruloplasmin, haptoglobin and α_1 -acid glycoprotein in goats with induced *Staphylococcus aureus* (Group I) and *Streptococcus agalactiae* (Group II) mastitis in order to verify its importance as early biomarkers of mammary infection in these species.

Materials and Methods: This experiment was approved by the Ethics Committee on Animal Use (CEUA) from UNESP, Brazil under protocol number 011878/11. GI was composed of five Saanen goats inoculated through the teat canal with about 9.5×10^9 colony forming units (CFU) of *S. aureus* and GII consisted of five French Alpine goats inoculated with approximately 4.4×10^{11} CFU of *S. agalactiae*. The sampling moment immediately prior to inoculation was designated M0 and then daily sampling was identified as M1, M2, M3, M4, M5, M6 and M7 when blood and milk samples were obtained. Serum ceruloplasmin, haptoglobin and α_1 -acid glycoprotein were obtained by the SDS-Page Electrophoresis technique. Milk samples were used to monitor the development of clinical mastitis through the strip cup test, California Mastitis Test (CMT) and microbiological analyzes. Data were subjected to analysis of variance and the means to Dunnett test, considering a 95% significance level ($p < 0.05$).

Results: It was noted that the experimental infection model developed for the induction of clinical mastitis in goats was effective in both groups. Serum increase percentages of up to 254% in GI and 112% in GII were obtained for serum Ceruloplasmin. Haptoglobin concentrations showed percentages of serum elevation of 444% as early as 48 hours after inoculation (M2) in GI, and 2,000% in M3 in GII, with the highest elevation percentage of 2,500% being observed in the last sampling moment (M7) in GII. Serum α_1 -acid glycoprotein concentrations did not differ significantly from initial concentrations in goats from GI until the last sampling moment (M7) when there was an 84% elevation percentage. In GII there was no significant elevation percentage obtained. In this group the highest value observed was at M5 sampling moment, when it was observed a 14% elevation percentage compared to M0.

Conclusions: Serum Haptoglobin was an early and reliable marker in the detection and monitoring of experimental mastitis, whether induced by *S. aureus* or *S. agalactiae*. Ceruloplasmin was a moderate marker and serum α_1 -acid glycoprotein showed limited significance, being a better marker of chronic rather than the acute process in *S. aureus* induced mastitis.

Small Ruminants

P01-001-158

Urinary Bladder Sonography And Antegrade Cystourethrography In Rams And Wethers With Obstructive UrolithiasisUschi Schwarz¹, Marlene Sickinger¹, Kerstin von Pückler², Kernt Köhler³, Martin Kramer²¹Clinic for obstetrics, gynecology and andrology with veterinary ambulance, ²Clinic for small animals (veterinary surgery), ³Institute for veterinary pathology, Justus-Liebig-University, Giessen, Germany

Objectives: Due to the predisposing anatomy of the urethra in small ruminants, obstructive urolithiasis continues to be a challenging problem with often unsatisfactory outcome. Several surgical techniques to restore normal urination in patients suffering from this disease have been described. Nevertheless, use of diagnostic measures (ultrasonography, cystourethrography) and longterm survival times are still rather limited. Therefore, diagnostic possibilities in cases of obstructive urolithiasis undergoing cystotomy versus laparoscopic tube cystostomy are described and outcome rates are compared.

Materials and Methods: Ultrasonographic examinations of the urinary bladder were performed in 26 male small ruminants suffering from obstructive urolithiasis and undergoing tube cystostomy via laparotomy (control group, n=13) or laparoscopy (test group, n=13) respectively. Horizontal and vertical diameters of the urinary bladder as well as a qualitative assessment of the bladder wall and its content were evaluated using a Mindray M5 (5.0 MHz). In patients with unresolved disturbance of urine passage after surgery antegrade urethrocytography was used to determine the localization of obstruction.

Results: Both surgical techniques used in this study resulted in a short term success rate of 69 %. Long term follow up for both techniques revealed a success rate of 46 %. Measurements of bladder diameters showed no significant differences between test and control group. In all patients presented with disturbed voiding ultrasonography displayed dilated urinary bladders with thin, hyperechogenic walls, hypoechogenic contents and in some cases small amounts of hyperechogenic sludge. Five out of the initially presented 26 animals showed persisting urethral obstructions after surgery. Due to lacking owner's consents, in only two patients an antegrade cystourethrography could be performed. In both cases filling defects in the area of the proximal flexura sigmoidea could be shown.

Conclusions: Success rates of both surgical methods are comparable to those already published, which continue to be unsatisfactory. To enhance surgical outcome new or modified techniques that are derived from small animal's surgery should be applied. Diagnostic measures including ultrasonography and antegrade cystourethrography are highly recommended as standard diagnostic procedures.

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Small Ruminants

P01-001-161

Supplementation with Vitamin "E" in Rambouillet Ewes during the Last Third of Pregnancy: Effect on Lamb Birth Weight

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Objectives: The transfer of nutrients from dam to its fetus occurs by placental transport. However, vitamin E (VE), which functions primarily as an antioxidant, does not cross the placenta in any appreciable quantity. Consequently, pre-partum supplementation of VE is essential to promote an optimum fetal growth while a prime birth weight (BW). The aim of this study was to evaluate the possible effect of supplementation of VE during the last third of pregnancy upon BW. Besides, the influence of some non-genetic effects of variation upon BW expression in Rambouillet lambs, was also evaluated.

Materials and Methods: The study was conducted at the Faculty of Agriculture and Veterinary Medicine, UASL, San Luis Potosi, Mexico (22°NL, 1,835 m). Multiparous pregnant Rambouillet ewes (n=37 ewes, 71.5±1.75 kg, LW, ≥ 3 yr. old) were mated to a single Rambouillet sire of proven libido and fertility; during the breeding period, the exact date of mating was recorded. Starting on the last third of pregnancy (d -50), ewes were randomly divided in two treatments: 1) Control (CON; n=17, no vitamin E treatment) and 2) vitamin E-supplemented (VE; n=20, receiving 4 IU vitamin E kg⁻¹ LW. VE-injections considered a commercial product and contained 27.2 IU mg⁻¹ of α-tocopherol (Lab-Torneo, Mexico). Intramuscular injections were given weekly on the same day of the week at 0800 h, from late gestation (d -50) up to lambing (d0); a total of 50 lambs at birth (5.32±0.26 kg) were considered for the analysis. The defined model considered to BW as dependent variable while experimental group (EG), ewe lambing number (NL), litter size (LS), and of sex lambs (SL) as fixed effects; the variables NL, LS and treatments were included as covariates. Lambs were weighed with a Torino® hanging scale with a capacity of 10 kg and a minimal graduation of 20 g. The health status of the experimental units was controlled by an experienced veterinarian during the whole experimental period; no health problems were observed during the trial. All the methods and management of the experimental units were in strict accordance with accepted international guidelines for ethical use, care and welfare of animals in research.

Results: Birth weight is one of the most important factors influencing the pre-weaning growth of the young and the onset of puberty; a heavier birth weight is an indication of better nutrition level of the dam while promotes reduction of perinatal mortality of the newborn. However, in our study, VE-supplementation during late pregnancy did not affect the birth weight of the progeny; the least square mean average for BW was 5.2±0.16 kg. Besides, no differences (P>0.05) occurred between males (5.20±0.23 kg) and females (5.06±0.26 kg). Yet, BW was affected (P<0.05) by NL and LS; sheep with three lambings had heavier lambs (5.38 ± 0.16 kg) regarding to ewes with 2, 4, 5 and 6 lambings (5.38±0.18, 5.44±0.38, 5.09±0.17 and 4.41±0.17 kg, respectively). Simple born lambs were heavier than double (5.67 vs. 4.67±0.11 kg). Some studies have reported that oral tocopherol supplementation during the last third of pregnancy in sheep increased the mRNA expression of interleukins and different angiogenic factors in uterus, caruncles and cotyledons, affecting in a positive fashion fetal growth. Yet, the lack of any effect of supplementation of vitamin E in late gestation on birth weight agrees

with other studies. Certainly, maternal undernutrition did not affect the progeny's birth weight reported in this study which is within the range of birth weight for the Rambouillet breed.

Conclusions: Long term supplementation with VE during the last third of pregnancy in Rambouillet ewes did not affect the expression of the lamb birth weight, yet, NL and LS affected the phenotypic expression of BW. Therefore, once adjusted for the non-genetic factors causing genetic variation in the BW phenotypic expression, BW should be considered as an important selection criterion in sheep breeding programs to improve production efficiency of the flock.

Small Ruminants

P01-001-162

Expression Of Metalloproteinases 2 And 9 In Sheep With Acute Lactic Ruminal Acidosis And Laminitis

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Objectives: The ruminal acidosis, either in acute or subacute form, is considered one of the main etiopathogenic causes of laminitis. The matrix metalloproteinases (MMPs) are secreted as pro-enzymes by neutrophils, monocytes and macrophages in response to various stimuli. The laminar foot degradation caused by MMPs has been associated with inflammatory response due to release of MMPs activating factors from the bloodstream into the digits, contributing to the degenerative process, shown particularly in horses. This study aimed to evaluate the expression of MMPs 2 and 9 in sheep with acute lactic ruminal acidosis (ALRA) experimentally induced.

Materials and Methods: Ten animals received 15 g/kg of body weight of sucrose orally, and the blood samples were taken at the following intervals: 4, 8, 12, 16, 20, 24, 28, 32, 36, 48, 72, 96, 120, 144 hours and on the second, third and fourth following weeks. The samples were separated into serum fractions by centrifugation at 2,500g for 10 minutes. The serum fraction was stored at -80 in sterile plastic Eppendorf microtubes of 1,5 mL, to perform the zymographic test. The data was submitted to analysis of variance with repeated measures in time, where the crop was the tested factor (17 levels), with the animals constituting the blocks (10).

Results: During the clinical manifestations of ALRA five animals had alternating support of forelimbs, reluctance to get around in lateral position for a long time, forelimbs sensitives to palpation and temperature increased on the corneal area, characterizing the process of acute laminitis that began between 24 and 28 hours and it was evident even at 72h after induction of ALRA. Only the pro-active forms of MMPs were expressed in zymography gels with higher values occurring mainly between 32 and 144 hours.

Conclusions: In horses is believed that MMPs are also causes of laminitis after episodes of acute abdomen. In ruminants it is believed that MMPs have the same dynamic that shown in horses, and the results obtained in this study demonstrate that these enzymes play an important role on the genesis of acute laminitis after ALRA.

Surgery

P04-004-192

Right Abomasal Displacement In Six Pregnant Cows From Northeastern Brazil

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Objectives: Right abomasal displacement (RAD) is a digestive disorder of high milk-yield cows and occurs mainly during the first six weeks post-partum. Therefore the occurrence during pregnancy is infrequent. This work aimed to report the clinical and laboratorial findings, and to evaluate the treatment choice in six pregnant cows with RAD.

Materials and Methods: Six 5 to 10-year-old crossbred cows were clinically evaluated due to sudden drop in milk yield, hyporexia and apathy. Clinical examination, hematological profile and ruminal fluid analysis were performed. Two moderate cases, without severe abdominal distention, appetite for roughage and metallic sound ("ping") reaching at the most the 8th intercostal space were treated conservatively, and four severe cases with moderate to severe abdominal distention associated to systemic disturbances were treated surgically by right flank pyloro-omentopexy.

Results: The management system was semi-confined with access to poor quality roughage and commercial bran (5-8kg/day). Pregnancy was accessed by rectal palpation and revealed two (n=2), five (n=1), six (n=1) and above eight (n=2) months gestation. Clinical evolution was 2-4 days. Clinical findings were apathy, dehydration, ruminal bloat, increased abdominal tension, "ping" varying from the 8th intercostal space to the right paralumbar fossa, splashing sound during right flank ballotement, and liquid blackish feces with mucus. Two cows also presented mastitis. Hematological exam was performed in three animals showing light leukocytosis (mean value: 13350/ μ L) by neutrophilia and hyperfibrinogenemia in one cow (1000mg/dL; reference: 400-700mg/dL). Ruminal fluid analysis showed compromised flora and fauna dynamics and increased chlorine ion concentration (mean value: 45,61 mEq/L). Four cows were surgically treated with a right flank pyloro-omentopexy, and two cows were clinically treated with transfaunation, dietary and hydro-electrolyte correction. All cows were discharged with no complications and deliver healthy calves.

Conclusions: Right flank pyloro-omentopexy showed to be a safe approach for the surgical treatment of RDA in pregnant cows, whilst the clinical protocol described was effective in treatment of light RDA when there is no systemic imbalance and the cow remain with appetite for roughage.

Surgery

P04-004-193

Use of bovine pericardium grafft for umbilical Hernioplasty in calf

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Objectives: This study aims to describe the surgical approach to Umbilical hernia using bovine pericardium graft on the hernia ring in a heifer that arrived at the veterinary clinical center of the University Centre of Patos de Minas (UNIPAM). The animal have eight months, breed cross, 150 kg and had recurrence of umbilical hernia about 4 months, recurrence hernia since after 10 days of first surgery had been performed.

Materials and Methods: To do the second surgical procedure the animal was fasting for 12 hours, it was sedated with a combination of 2 mg / kg ketamine and 0.1 mg / kg xylazine 2%. Trichotomy was performed in the abdominal area and then antiseptis with chlorhexidine 2%. Local anesthesia around the hernia sac was with lidocaine epinephrine, 7 mg / kg. The heifer was placed on the operating table in ventral position and incision was about 15 cm in the midline, under hernia ring. Was remove adhesions of hernia and place suture cross mattress for closing hernia ring use polypropylene 2. The graft was stored in 98% glycerol for superior time than 15 days. Glycerol to be an excellent antiseptic and low antigenicity. Before use, bovine pericardium it was washed with 0.9% NaCl solution then left to hydrate in this solution for 30 minutes. The graft was cut as needed size and abdominal wall was scarified to increase grip. Then the pericardium was set 2 cm extending beyond the edges of wound, with nylon 2-0 and polyglycolic acid 1 used interchangeably with separate mattress suture, moreover, it was fixed to allow slight elasticity of abdominal muscles and prevent breakage of attachment points. The skin suture was performed with nylon number 0 place suture separate mattress. Pain control was performed 1.1 mg / kg flunixin meglumine for three days and antibiotic therapy ceftiofur sodium dose of 2.2 mg / kg for 5 days every 24 hours. Surgical wound cleaning was performed daily with chlorhexidine 2%.

Results: To obtain Rapid Recovery animal remained eight days with suspensory tissue with little pressure to diminish edema, protect against dirtiness and insects. The Heifer don't have any significant edema in surgery, no infection in suture and nule reaction with bovine pericardium graft. With fifteen days the skin points were taken of. Regarding clinical response to treatment and rapid recovery, by immediate surgery and pos-operative care, the animal returning to routine of production system with 5 days.

Conclusions: Efficient surgery without contamination is important to avoid umbilical hernia recidive. In animals with thin abdominal wall is important use any graft in hernioplasty. Bovine pericardium graft is good option to use because is very resistant, cheaper and no rejection in bovines.

Surgery

P04-004-194

A case study of the hindlimb amputation in a Holstein cow

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Objectives: A Leg amputation is a localized treatment that is effective and appropriate as remedial or palliative treatment for external trauma or malignant tumours such as osteosarcoma and mastocytoma, as well

as congenital deformities. However, in cows, this procedure has rarely been performed because of factors such as the feeding environment and economic viewpoint. In the present case, we performed amputation in a cow with an open fracture in the tibia while she was in the midterm of her pregnancy. We maintained her pregnancy until the last term, and she progressed until delivery of the calf by caesarean procedure.

Materials and Methods: The subject was a 30-month-old Holstein cow raised in a dairy farm in the Kanagawa prefecture experiencing her first pregnancy. She was pushed by another cow during free-range feeding and fell down, and it was found that the inside of her lower left limb was injured and bleeding. Because her condition gradually worsened even with continued antibiotic therapy, she was admitted to the Azabu University Animal Hospital for diagnosis and treatment. At the time of admission, her weight was 430 kg; she was 5 months pregnant and unable to stand. Her left lower hindlimb moved abnormally, and we found an open wound in the inner portion of her left lower hindlimb. The fracture was examined by palpation, and was diagnosed as an oblique fracture of the left tibia on radiography.

Results: Because of the position of the fracture and the cow's weight, it was judged that repositioning by external fixation as well as internal fixation would be difficult. We performed the amputation procedure on the day after delivery in order to maintain her pregnancy. The distal end of the fractured tibia was severed at the point of the fracture, and the proximal end was removed from the femur at the knee joint. We covered the surface of the femoral block with the surrounding muscle tissue. Subsequently, we closed the subcutaneous tissue and skin in the usual manner. Postoperatively, we continued antibiotic treatment for several days to prevent infection. It was difficult for the cow to stand on her own immediately after the surgery, but from the following day, she was able to stand with the aid of a cow lift. Gradually, the amount of time she was able to stand increased. Approximately 3 weeks after the amputation, she was able to stand on her own. However, after the 10th week, the amount of time she was lying down increased. From the 12th week onwards, she once again was unable to stand. Because of her deteriorating condition indicated by the inability to stand, we delivered her calf by caesarean procedure as she entered the 9th month of her term.

Conclusions: In the present case, worsening of the overall condition was slight, and no other injuries were found apart from the tibial fracture; therefore, we performed amputation with the goal of maintaining the pregnancy. At approximately 3 weeks after the operation, it was necessary to assist the subject in standing up. However, after some time, she was able to stand on her own. Therefore, use of the amputation procedure may be possible in cows if their weight is relatively less and if they are in the mid-to-last term of their pregnancy, as in the present case.

Surgery

P04-004-195

Middle line laparotomy in calf with trichobezoar in rumen

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Objectives: This study aims to describe surgical approach of exploratory laparotomy, followed by rumenotomy in calf for removal trichobezoar located in rumen. The calf has 75 days and arrived in veterinary clinical center of UNIPAM with depression, acute abdominal pain and milking disinterest in last 5 hours.

Materials and Methods: Anamnese animal had tachycardia, tachypnea and slightly tympanic rumen. Hematology analysis had low hematocrit 16.89% and thrombocytopenia 85000 / mm³. Abdominal x-ray was performed in positions lateral and ventro-dorsal and observed a rounded mass in rumen region. Exploratory laparotomy was did to remove the mass and check if have another foreign body. For surgical procedure the animal was sedated with 2 mg / kg ketamine and 0.1 mg / kg xylazine. Anesthesia was maintained with isoflurane, fluid therapy with Ringer lactate and performed blood transfusion 1000 ml. Exploratory laparotomy was in midline incision 8 cm after xiphoid process and 5 cm caudal to umbilicus. Just was identified foreign body in rumen. Rumenotomy was remove trichobezoar about 8 cm of diameter, that was occupying all rumen. The rumen was closed wired polyglycolic acid number 0. Suture was performed in two plans simply continues and another invaginating, Cushing. It was subsequently washed with solution prepare with 1 l of sodium chloride 0.9% plus dimethylsulfoxide 10 mL and plus 4000 mg amikaciana. This solution was placed intraperitoneal too before midline closure. The middle line was closed with polypropylene number 2 wire at cross mattress suture. The skin suture was performed with nylon number 0, intercalating horizontal and vertical separate mattress points. For pain control was administered 1.1 mg / kg of flunixin meglumine every 24 hours during 3 days. And antibiotic therapy with ceftiofur 2.2 mg / kg every 24 hours for 10 days and 4.4 mg gentamicin / kg every 12 hours during 3 days.

Results: Animal returned anesthesia without abdominal pain and go back to milking and feed in 3 hours after surgery. In 48 hours the calf is milking the same volume as before problem. The animal remained eight days with suspensory tissue with little pressure to diminish edema, protect against dirtiness and insects. With fifteen days the skin points were taken of. Regarding clinical response to treatment and rapid recovery, by immediate surgery and pos-operative care, the animal returning to routine of production system with 15 days.

Conclusions: The solution with antibiotic placed intraperitoneal and suspensory tissue is helpful to pos-operative success. But most importante to avoid animal death is perform rapid diagnosis and immediate surgery to remove trichobezoar.

Surgery

P04-004-196

Operative treatment of abdominal wall hernia in a newborn Simmental calf

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Objectives: The purpose of this study was to investigate an unusual case of abdominal wall hernia in a newborn calf. A one-week-old Simmental calf was admitted to the Large Animal Hospital of the Veterinary Faculty of Erciyes University with a story of an abnormal body posture that involves the entire abdomen.

Materials and Methods: The detailed examination began with observations of the calf from a distance and is followed by a physical examination of the abdomen to assess the developing abdominal hernia. Additionally, blood samples were collected from jugular vein of newborn calf for hematology and blood gas analysis. Abdominal ultrasound was used to look at organs in the abdomen, including the liver, gallbladder, spleen, pancreas, kidneys and intestines. Radiography was performed in a latero-lateral position of the patient.

Results: In this case, a lateral hernia due to a weakness or defects in the fibromuscular tissue (fascia and muscle) of the right abdominal wall, resulting in a protrusion of abdominal contents, was diagnosed by physical examination. It caused a noticeable bulging, while all the other clinical and laboratory parameters remained unchanged. Localization of the abdominal wall defect was confirmed with abdominal ultrasonography and radiography. Because the bulge could not be reduced, surgical repair was decided. A polypropylene mesh sack of 15 x 15 cm is sewn around the abdominal wall hernia on the right side.

Conclusions: After surgical intervention, the intestines or other abdominal organs were not damaged and the newborn calf did not show any signs of digestion problems. This case indicates that abdominal wall hernia resulting from defects in calves can be surgically treated successfully.

Surgery

P04-004-197

General Inhalational Anesthesia due to Emergency Ventral Hernia Surgery in Calf

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Objectives: General anesthesia is rarely carried out in these species especially in calves because of susceptibility to complications associated with recumbency and anesthesia: regurgitation, tympani, hypoxia, apnea, aspiration pneumonia and trauma as well. The most common surgical procedures are undertaken using paravertebral nerve block or local infiltration anaesthesia.

Materials and Methods: Three weeks old calf was brought to the Large Animal Clinic suffers from severe abdominal pain, apathy and lack of appetite and urination with large evident abdominal wall deformation. The clinical examination was done. The body temperature was increased up to 40,0° C, HR 130/min, BR 45/min and severe abdominal pain was confirmed especially near the abdominal wall deformation, which was swollen, hot and hard to palpate. Ultrasound examination of the abdomen was performed. The ventral hernia was diagnosed with large intestine section presence inside hernia sac. The general inhalational anesthesia (sevoflurane in oxygen; Sevoflurane Baxter) with endotracheal intubation following intramuscular premedication (medetomidine 0,002 mg/kg; Cepetor 1mg/ml CP-Pharma Germany, ketamine 0,5 mg/kg Bioketan 50 ml Vetoquinol Biowet PL) and intravenous induction (propofol 5mg/kg; Scanofol 10mg/ml ScanVet Poland) was performed due to hernia surgery. The ventral hernia was removed including bowel part resection.

Supportive therapy includes patient positioning, fluid administration and continuous monitoring techniques was applied from pharmacological sedation until full the very fast recovery. The postoperative antibiotic (penicillin with dihydrostreptomycin; Shotapen Virbac; 1ml/10 kg) in combination with non-steroidal anti-inflammatory drug (flunixin 2,2 mg/kg; Flunimeg Scanvet Poland) was administrated for 5 following days.

Results: The maintenance of general anaesthesia (sevoflurane in oxygene) was smooth and easier to control compared with general anaesthesia maintenance with isoflurane in oxygene. No complications like heart arrhythmias, respiratory depression and anaphylactic reaction were observed during surgery. The recovery process after endotracheal tube removal was much faster (5 min- standing position) without complication in intensive care room for large animals compared with recovery process after isoflurane administration (15 min). The back to consciousness after sevoflurane anaesthesia is faster than after isoflurane anaesthesia which is preferably especially in ruminants. After sutures removal it returns to the farm.

Conclusions: General inhalational anesthesia (sevoflurane in oxygene) allows for optimal however safely, painless management of the emergency surgeries in calves. Furthermore, the medical sciences state of art progress affords application of the latest anaesthetic drugs and techniques allowing to minimize anaesthetic risk, pain and distress of the animals in particular with poor clinical status. Sevoflurane is suitable inhalation anesthetic for use in calves especially for emergency however present cost is limiting factor for its use in food animals at regular base.

Surgery

P04-004-198

One-step laparoscopy for correction of the left abomasal displacement in high-yield Holstein dairy cows in Brazil

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Objectives: Surgical procedures for correction of abomasal displacement have been one of the most frequently performed in dairy cows, with many surgical techniques described for treatment since the first disease cases in the 1950s. Although with no report to date in Brazil, the one-step laparoscopy technique has associated advantages of traditional technique, such as abdomen visualization, with minor trauma of minimally invasive techniques.

Materials and Methods: Accordingly, one-step laparoscopy seconding Christiansen and Barisani was applied to 21 high yield dairy Holstein Friesian cows from two dairy region of Parana State with left abomasal displacement. In cows heavier than 700 Kg, a third access, over the 10° or 11° intercostal space, 15cm ventrally of second access, has been used to introduce the working channel and the big needle, because the device could not reach the right paramedian side in large cows. Short-term efficacy (during one month) of one-step laparoscopy abomasopexy has been evaluated. The surgeries have been occurred between April/2014 to April/2015. The diagnosis of LDA has been established through clinical examination in addition to the presence of scant and diarrhetic faeces, dehydration and tympanic and metallic ping on auscultation-percussion in the left para-lumbar fossa

Results: Overall, mean duration of surgery was (without animal's preparation) 35.04 ± 11.40 minutes (range, 20 to 60 minutes). Three animals underwent the Barisani (2004) technique. However, some complications were found, as exteriorization of only on toggle thread at the moment of right paramedian perforation and toggle bar suture lodged subcutaneously. Furthermore, impaired thread sliding off the trocar occurred because of friction between knot joining threads and big needle. Therefore, the surgery method has been changed to Christiansen (2004) technique. In 12/21 (57.14%) cows, this technique was performed with no complications. Ruminal (four animals), abomasal (two animals), both (one animal) and spleen (one animal) perforations occurred during surgery procedure but without post-operative intercurrent. One cow has been showed pyloric obstruction caused by toggle bar suture, but early removal restored abomasal flux. Three animals died in consequence of different causes. One cow has showed recurrence of displacement on month following surgery. Third access was efficient in correct abomasal displacement in cows heavier than 700Kg.

Conclusions: One-step laparoscopy abomasopexy proved to be efficient in correcting LDA under field conditions. Complications found were also reported in the literature, although they have not interfered in operation efficiency. Despite the equipment price, the possibility of carrying out a procedure faster, safer and less traumatic for the cow are strong arguments for the adoption of the technique by the bovine practitioner.

Sustainable Agriculture

P02-002-207

Analysis of bovine production dynamics related to soybean, sugar cane and corn production in Brazil

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Objectives: The objective of this study was to analyze the spatial-temporal dynamic in Brazil's cattle production during the period from 1978 to 2012 related to soybean, sugar cane and corn production national. In addition, we analyzed the substitution and association processes between these systems using correlation analysis between the temporal series of production.

Materials and Methods: In the present study were considered Municipal production data of the effective cattle herd in period between 1978 to 2012 and the amount produced in tons of soybean, sugar cane and corn from 1993 to 2012, both acquired from Brazilian Institute of Geography and Statistics web page. Temporal series were used to calculate growth and acceleration rates for each production, as well as the direction tendencies and inverse correlations between the soy and beef production, as also the sugar cane and beef production in Brazil. The production data was organized in a table and spacialized on map of Brazil from ArcGIS® program.

Results: The results showed high growth rate and acceleration of soybean and corn production in the South, Midwest and the western of the Northeast region, with a tendency of these productions moving to the northeast of the country. The South-Central region achieved high growth rates and acceleration of sugarcane production, also was observed the displacement of sugarcane production from the Northeast region to the South-Central region, indicating the loss of participation in the Northeast over the analysis period. Meanwhile, in the recent period from 2003 to 2012, the cattle production showed high growth rate and acceleration of production in the Legal Amazon area. The inverse correlation between cattle production and sugarcane production from 2002 to 2012 was seen in municipalities in the South-Central region. Moreover, most of the municipalities that showed an increase in sugarcane production and decreased in cattle production were in this region. The inverse correlation between the production of soy and beef production was seen mainly in municipalities in the Midwest, Southeast and South. The entry of flex-fuel cars in the Brazilian market in 2003 and the rise of commodity prices in the international market can explain the recent decline in beef production in the Midwest, South, Southeast, led by increased production of soybean and sugarcane in those regions, shifting beef production to areas further north. The causes of displacement of these productions are complex, which may be linked to climatic, topograph, access to credit and technical assistance as well as market issues.

Conclusions: The methodology used was efficient in multi-temporal analysis of sugarcane, corn, soybean and cattle production, promoting the understanding of the dynamics of the productions in Brazil, which can be used as a subsidy to public policies. Long-term public policies are necessary to understand these dynamics in an integrated management to generate improvements toward sustainable practices intensification of land use, taking into account regional disparities and promoting science and innovation in Brazilian agriculture.

Sustainable Agriculture

P02-002-208

Regional Changes in Brazilian Beef Cattle Production

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Objectives: The objective of this study was to analyze the dynamics of cattle production in Brazil on a municipal scale and to identify potential areas of strangulation for continued growth and variables that could limit the increase in production and productivity.

Materials and Methods: This study considered all municipalities in five regions of Brazil (5561). Data on cattle production in Brazil were obtained from the website of the Brazilian Institute for Geography and Statistics from 1977 and 2011. The spatial midpoint of production in the country was calculated for each year to assess the direction of production in the country. Changes in the relative production and acceleration of production were calculated, and these data were spatialized using ArcGIS®. Cluster and canonical discriminant analyses were performed to further highlight differences between regions in terms of cattle production.

Results: Currently, the mean production point has moved from the Center of Minas Gerais State (in the southeast region) to the North of Goiás State (in the midwest region). These data generate a reflection of changes in environmental factors, such as pasture type, temperature and humidity. The relative increase in production in the midwest and northern regions is evident, which is considerably higher compared to other regions. In the northern region, because the original herd was small, the initial acceleration (1980-1990) was high with the migration of cattle production from other regions, but in numeric terms, the herd size has only become expressive within the last 10 years. Recently, "traditional" cattle-rearing regions, such as the south and southeast, showed a reduction in growth rates as well as a reduction in herd size or internal migration over the period studied. Regardless of the slowing growth and significant reduction in herd numbers, the states of São Paulo, Minas Gerais, Mato Grosso do Sul, Mato Grosso and Goiás, still represent the majority of beef exports in Brazil. These states are where the main structure for export of slaughtered animals is located, and they are also the main centers for completing feed-lots, which explains the fact that even with a reduction of the herd, they remain the leading exporters of meat. However, with the technological improvements in the agricultural sector, soils that are currently limited for farming should, in the future, present economic viability and force livestock production to new regions of the country.

Conclusions: Changing the configuration of a production system for beef cattle production from one region to another requires skills and expertise as it passes through a redefinition of farming objectives, as well as strategies and processes to achieve better results. Future studies should incorporate variables related to the expansion of crops, income from plant products, average area of farms, different specializations in cow-calf, growth and completion of production systems and density of cattle in different regions of Brazil.

Sustainable Agriculture

P02-002-210

Nitrogen Efficiency as weak Point analyze in organic dairy farming

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Objectives: The aim of the current study is to clarify how weak points analyze of nitrogen cycles in organic dairy farms enables farm managers to identify the points in the farm nutrient cycle at which nitrogen leaching is most critical.

Materials and Methods: The subject of the investigation is of the Educational and Research Farm at the Justus-Liebig-University Gießen, Gladbacherhof. The investigation period was from 1993 to 2006. The corresponding data basis were the farm accountancy, the Acreage index, the milk recording data, feed analyzes and yield. Different nutrient cycle system levels have been observed. Every level was analyzed by special nutrient balances. The efficiency is expressed as Input:Output-ratio in percent. If one level has an input of 100 units nitrogen and an usable output of 50 units nitrogen it means an N efficiency of 50 %. Based on a top down decision tree the weak points were systematically identified.

Results: Based on the farm gate balance a nitrogen efficiency of 53 % in the average of three years was observed. In the next step the livestock level was analyzed. The nitrogen efficiency on the dairy production level (feedstuff input to the stable vs. milk,meat and pasture output) amounted 98 %. So this level was excluded as weak point in this farm system. The analyze of the cropland and grassland balance confirmed a nitrogen efficiency of 75 % on grassland, but only 49 % on the cropland. While at the dairy production the nitrogen utilisation is very good, the success stayed on the cropland off yet. The low nitrogen efficiency of only 49 % is associated with a lost amount of reactive nitrogen.

Conclusions: In the present study it can be resumed that weak points analyze is able to identify critical points in the nitrogen cycle of a farm system. The analyse has to go on ...deeper“ levels of the nutrient cycle.

Therapeutics & Pharmacology

P04-004-106

Brucellosis in buffaloes: A novel treatment approach using chemo-immunotherapyHaisem U. Rasool¹Muhammad Avais¹Muhammad S. Khan¹Masood Rabbani²Khushi Muhammad³

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Objectives: Brucellosis in cattle and buffaloes is caused by *Brucella abortus* and characterized by late term abortions, placentitis, retained placenta, still birth, orchitis and infertility imposing colossal economic losses to the dairy industry. It is a zoonotic problem that need to develop an effective treatment/control strategy. Therefore present study was carried out to evaluate a novel treatment approach for brucellosis in buffaloes using chemo-immunotherapeutic agents.

Materials and Methods: Experimental study included healthy (A and B groups) and brucellosis positive (C and D groups) buffaloes. Each of the group A, B, C and D contained 5 animals. The animals in both the groups A and C were given 1) four injections of long acting oxytetracycline (20mg/kg body weight; BW) intramuscularly (IM) repeated after every 48 hours, 2) five consecutive injections of streptomycin (13mg/kg BW IM), 3) three consecutive injections of flunixin meglumine (2mg/kg BW IM) for three days followed by five injections (IM) of selevit for five consecutive days and 4) combined vaccine (oil based chemically inactivated FMD virus and live attenuated rough strain of *B. abortus* RB51). Each animal of group B and D served as negative and positive control, respectively. Effectiveness of a particular treatment protocol was evaluated on the basis of antibody titer (RBPT and ELISA) and antigen detection thorough PCR. Moreover, birth weight and placenta dropping time in forthcoming parturition of the animals was also recorded

Results: Each animal of the treated groups were negative to enzyme linked immunosorbent assay (ELISA) on six months post-treatment and polymerase chain reaction (PCR) on subsequent parturition. On screening through RBPT and ELISA, the treated and immunized animals showed increase in ELISA antibody titer against *B. abortus* for certain period of time but subsequently became negative. Antigens of the pathogenic *B. abortus* inactivated either by antibiotics or gamma interferon activated macrophages in the presence of their specific memory B cells may be responsible for mounting the ELISA or RBPT antibody titer and declining thereafter on clearance of the *Brucella* specific antigen from the host body. On subsequent parturition, the birth weight of calves and placenta dropping time were non-significantly different ($P>0.05$) in brucellosis negative buffaloes and buffaloes recovered from brucellosis in response to treatment

Conclusions: From the results of this study it was concluded that use of oxytetracycline in combination with streptomycin, flunixin meglumine, vitamin E + selenium followed by oil based FMD and RB51 vaccines (chemo-immunotherapy) is an effective way to treat *Brucella* infected buffaloes in local circumstances.

Comments: Brucellosis is serious problem to dairy herds in Pakistan and it is almost impossible to implement test and slaughter policy due to poor economic status of the farmers. Therefore, an attempt was made to treat brucellosis, which showed promising results.

Therapeutics & Pharmacology

P04-004-107

Blood Electrolytes Of The Calves Treated To Neonatal Diarrhea With Fast Action Enrofloxacin And Oral Electrolyte And Energy ReplacementViviane R. Rabassa¹Ismael M. Cavazini¹Luis Gustavo C. da Silva²Alexandre Bilhalva¹Camila Pizoni¹Rubens A. Pereira¹Augusto Schneider¹Eduardo Schmitt¹Marcio N. Corrêa¹ and Center for Research, Teaching and Extension in Animal Science (NUPEEC)

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Objectives: The aim of the study was to evaluate the effect of the therapeutic strategies for the treatment of neonatal diarrhea using the fast action enrofloxacin and its association with oral electrolyte and energy replacement on blood electrolytes (sodium and potassium) of the Holstein calves.

Materials and Methods: This study was conducted in a commercial farm located in southern Rio Grande do Sul - RS - Brazil (32.8 ° 16 'S, 52.8 ° 32'L). Were used 24 Holstein calves, monitored from birth to sixth week of life. During the experimental period were performed clinical tests twice weekly on all animals. After showing diarrhea clinical signs, the calves were randomly assigned to the treatments: Control Group, with animals remained healthy (CONT; n = 6); Group Antibiotic (ATB; n = 4) Group Antibiotic + Intravenous support (ATB + IS; n = 4); Group Antibiotic + Oral support (ATB + OS; n = 7); Group Intravenous support (IS; n = 3). The active ingredient used in the groups that received antibiotics was the fast action enrofloxacin (Kinetomax®, Bayer Animal Health, Germany), a single dose of 7.5 mg/kg of body weight (BW), intramuscularly (IM). The support used in the treatment ATB + IS and IS groups consisted of oral administration of activated charcoal (6 g) once daily for 3 days, flunixin meglumine (Flunamine®, Bayer Animal Health, Germany) at a dose of 1,1 mg/kg of body weight, intramuscularly, and in cases of dehydration, intravenous fluid NaCl 0.9% based on the volume to be established according to the degree of dehydration. For ATB + OS group has been replaced by the IV fluid supplementation with oral replacement solution (Glutellac®, Bayer Animal Health, Germany) at a dose of 50 ml, added to milk, twice a day. After the diarrhea diagnostic was collected blood sampling at 0, 24, 72 and 120 h in relation to the diagnosis, to blood evaluation of the sodium and potassium.

Results: Serum sodium levels differ between groups (CONT: 206.40 ± 5.23 mmol/L; ATB: 172.44 ± 6.41 mmol/L; ATB + IS: 137.81 ± 6.41 mmol/L; IS: 181.80 ± 7.40 mmol/L; ATB + OS: 201.56 ± 4.85 mmol/L; $P<0.0001$), being the ATB + OS was the single able to maintain sodium levels similar to the CONT; the other groups differed from the CONT and ATB + OS group ($P<0.05$). Regarding the potassium levels was difference between groups (CONT: 5.12 ± 0.31 mmol/L; ATB: 5.84 ± 0.37 mmol/L; ATB + IS: 5.32 ± 0.37 mmol/L; IS: 6.18 ± 0.43 mmol/L; ATB + OS: 6.56 ± 0.28 mmol/L; $P=0.01$), being the IS and ATB + OS differed from the CONT group; the ATB + OS group also differed from ATB + IS group.

Conclusions: Thus, we conclude that treatment with fast action enrofloxacin and its association with oral electrolyte and energy replacement was effective in maintaining sodium levels avoiding hyponatremia caused by diarrhea, and increase potassium levels, due to the presence of this mineral in their composition. Since the hyperkalemia observed in untreated animals with fast action enrofloxacin and receiving only intravenous support, indicating that these animals had greater disease severity, making potassium release into the blood by acidosis and inflammation.

Therapeutics & Pharmacology

P04-004-108

Pulmonary lesions and clinical disease in *Histophilus somni*-challenged calves treated with, either tildipirosin or tulathromycin

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Objectives: To determine the efficacy of tildipirosin or tulathromycin metaphylaxis during early *Histophilus somni* infection of the respiratory tract in cattle as a model for bovine respiratory disease reduction

Materials and Methods: On study day 0, 24, 4-month-old dairy steers, negative for anti-*H. somni* antibodies and BVDV, were injected subcutaneously as follows: Group 1- Tildipirosin at 4 mg/kg; Group 2-Tulathromycin at 2.5 mg/kg; Group 3-NaCl, 1 mL isotonic saline/45.4 kg. Immediately after treatment, all were inoculated intrabronchially with 7.0 x 10⁹ CFU virulent *H. somni* strain 7735, which is susceptible to both antibiotics. Body weights, rectal temperatures, and clinical evaluations (0-9 total score) were recorded for time 0, 12 hours after inoculation, twice daily on days 1 and 2 and morning of day 3. On day 3, all animals, were sedated and humanely euthanized. At necropsy, lungs were weighed, percent pneumonia recorded, and tissue submitted for histopathology and bacterial isolation. Lung histopathology (LHS) and necrosis scores were determined for each lung. Statistical comparisons of clinical scores, rectal temperature, lung scores, and histopathology scores were analyzed by either non-parametric or general linear model methods.

Results: After challenge, weight losses per group were not statistically different (p=0.98). By 8 h, rectal temperatures and clinical scores increased from time 0 for each group (p<0.05), and increases were not significantly different among treatment groups (p>0.05). At necropsy, lung weight as a percentage of body weight was significantly higher for saline-treated than for tildipirosin-treated calves (p<0.01); however, there were no significant differences between the two macrolide-treated groups (p=0.50) or between tulathromycin- and saline-treated calves (p=0.34). Lung lesions varied from multifocal to diffuse, red and gray consolidation with or without obvious foci of necrosis. Fibrinous pleuritis was present in severely affected lungs. Percent lung consolidation ranged from a mean of 5.6%, 8.6%, or 18.2% among the tildipirosin-, tulathromycin-, or saline-treated groups, respectively. Percent lung consolidation in tildipirosin-treated calves were significantly lower than in saline-treated calves (p<0.02). There were no significant differences between the two groups of macrolide-treated calves (p=0.89) or between tulathromycin- and saline-treated calves (p=0.25). Histopathology showed , right lung lesions, necrosis, and LHS scores were significantly lower (p<0.05) for tildipirosin and for tulathromycin treatment compared to control. Necrosis was not found in any macrolide-treated lung. Bacterial isolation scores were significantly higher for the control than for tildipirosin and tulathromycin -treated (p<0.05) calves. *H. somni* was not isolated from tildipirosin-treated calves.

Conclusions: At necropsy, tildipirosin-treated calves had less lung consolidation and lowest relative lung weights than control calves. Histopathology scores of inflammatory changes were not significantly different for tildipirosin- and tulathromycin-treated calves. In the present study, necrosis was completely absent in lungs from macrolide-treated cattle. The significance of absence or reduced lung necrosis prevents the formation of scar tissue and allows the airways to be repaired through reepithelization. Thus, if necrosis can be avoided or reduced through macrolide treatment, this improves animal performance and welfare.

Therapeutics & Pharmacology

P04-004-109

Effects of tilmicosin on bovine neutrophil and lymphocyte functions

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Objectives: *Mycoplasma* species are highly contagious pathogens that cause mastitis, otitis media, arthritis, and pneumonia. As there is no effective vaccine against *Mycoplasma* infection, control depends on good husbandry and antibiotic therapy. Tilmicosin is a macrolide antibiotic used to control *Mycoplasma* infection. Macrolide antibiotics have recently been reported to modulate the immune system. However, the effects of tilmicosin on the bovine immune system have not been clarified. We examined the effects of tilmicosin on bovine neutrophil and lymphocyte functions for *Mycoplasma* eradication.

Materials and Methods: (1) Blood samples

Blood samples (30 mL each) were drawn from the jugular veins of five 3–5-year-old clinically healthy Holstein cows into heparin-containing tubes (20 IU/mL).

(2) **Isolation of neutrophils and lymphocytes:** Neutrophils and lymphocytes were isolated from heparinized blood by Ficoll-sodium iohalamate gradients (specific gravity, 1.078). The resulting cell population comprised >95% neutrophils and >98% lymphocytes, as determined by Wright-Giemsa staining; >99% of the cells were viable when assessed by trypan blue dye exclusion. Cells were treated with tilmicosin at concentrations of 0.01, 0.1, 1, 10, and 100 µg/mL.

Chemiluminescent (CL) response: 100 µL of neutrophil suspension (2 × 10⁶ cells/mL) were incubated at 37°C for 10 min in a luminometer (ATTO Co., Tokyo, Japan); 10 mL of luminol (final concentration, 10⁻⁴ M) was then added. After 5 min of equilibration, *Mycoplasma bovis* was added. The peak response and integrated value were evaluated.

(3) **Apoptosis:** 500 µL of neutrophil suspension (2 × 10⁶ cells/mL) were incubated with *M. bovis* (multiplicity infection [MOI], 100) at 37°C for 30 to 60 min. Annexin V and propidium iodide-positive cells were identified as apoptotic cells with a flow cytometer (Millipore Co., Tokyo, Japan).

(4) **Blastogenesis:** 200 µL of lymphocyte suspension (2 × 10⁶ cells/mL) and *M. bovis* (MOI, 100) were co-incubated at 37°C for 72 h. Blastogenesis was evaluated with the Cell Counting Kit 8 (Dojindo Co., Kumamoto, Japan).

Results:

1. LDCL response of neutrophils

Effects of tilmicosin on *M. bovis*-induced luminol-dependent CL (LDCL) responses of neutrophils were evaluated in lactating cows. Mean (standard deviation [SD]) *M. bovis*-induced peak CL values of neutrophils at tilmicosin concentrations of 0.01, 0.1, 1, 10 and 100 µg/mL were 162.1 (45.3), 215.5 (46.2), 254.2 (37.8), 255.4 (42.4), and 306.1 (55.9), respectively; these were significantly (p<0.01) higher than those of controls. Similarly, mean (SD) *M. bovis*-induced integrated CL values at tilmicosin concentrations of 0.01, 0.1, 1, 10, and 100 µg/mL were also significantly (p<0.01) higher than those of controls.

2. Apoptosis of neutrophils

Effects of tilmicosin on *M. bovis*-induced apoptosis of neutrophils were evaluated. Mean percentages of *M. bovis*-induced apoptosis of neutrophils at tilmicosin concentrations of 0.01, 0.1, 1, 10 and 100 µg/ml were almost the same as those of controls after 30 min of incubation. However, mean (SD) percentages of *M. bovis*-induced apoptosis of

neutrophils at tilmicosin concentrations of 0.1, 1, 10, and 100 µg/mL were significantly ($p < 0.01$) higher than those of controls after 60 min of incubation.

3. Blastogenesis of lymphocytes

Effects of tilmicosin on *M. bovis*-induced blastogenesis of lymphocytes were evaluated in lactating cows. Mean (SD) values of *M. bovis* induced-blastogenesis of lymphocytes at tilmicosin concentrations of 0.01, 0.1, 1, 10, and 100 µg/mL were 2.21 (0.23), 2.12 (0.32), 1.94 (0.42), 1.94 (0.42), and 1.82 (0.32), respectively; these were significantly ($p < 0.01$) higher than those of controls.

Conclusions: In this study, we clarified that tilmicosin enhances *M. bovis*-induced neutrophil and lymphocyte functions. Tilmicosin induces apoptosis of neutrophils stimulated with *M. bovis*. Neutrophil apoptosis provides anti-inflammatory benefits and may prevent tissue injury from the excessive inflammatory reaction caused by *M. bovis*. These results indicated that tilmicosin has therapeutic activity against *Mycoplasma* infection. We conclude that tilmicosin has not only bactericidal activity but also immunomodulatory and anti-inflammatory activity against *M. bovis*.

Therapeutics & Pharmacology

P04-004-110

Pharmacokinetic evaluation of a new combination of Fipronil and Fluazuron topically administered (pour-on) in cattle.

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Objectives: This study aimed to determine the pharmacokinetic parameters of a new combination of Fipronil and Fluazuron, administered to cattle via pour-on at a single dose of 1ml / 10 kg body weight (corresponding to 1.25 mg / kg of Fipronil and 2.5mg / kg of Fluazuron).

Materials and Methods: Eight crossbred animals of 14 to 24 months old (4 males, 4 females) were selected. Animals were not exposed to any other parasiticide at least 60 days prior to the start of the study. All animals received the combination product on day 0. A 10 mL blood sample was collected by jugular vein from all animals at time 0 (immediately before treatment) and with 3, 6, 12, 24, 48, 60 hours and 3, 4, 6, 8, 10, 12, 14, 17, 21, 28, 35 and 42 after treatment. Up to 2 hours after each collection, samples were centrifuged for 15 minutes at 1.200G. The separated plasma was placed into two siliconized Eppendorf® tubes (1.5 mL each) pre-identified and stored at -20°C until transportation to the laboratory. The analysis of Fipronil and Fluazuron were performed by liquid chromatography attached to a mass detector (LC-MS/MS). The following pharmacokinetic parameters were calculated from the plasma **Results:** elimination half-life ($T_{1/2\text{el}}$); maximum plasma concentration (C_{max}); time of maximum plasma concentration (T_{max}) and area under the curve (AUC).

Results: The Fipronil maximum concentration was 73.7 g / L (C_{max}) with 2.5 d after administration of the product (T_{max}). The $AUC_{42\text{d}}$ for Fipronil was 35699.4µg-hr / L and the half-life ($T_{1/2}$) was between 17 and 21 d after treatment ($C_{1/2} = 36.85\mu\text{g} / \text{L}$). For Fluazuron, the maximum concentration in the plasma was 26.1µg / L (C_{max}) occurred after 6 days after the product was administered (T_{max}). The $AUC_{42\text{d}}$ was 10472.6 4µg-hr / L. $T_{1/2}$ was 13.05µg / L ($C_1 / 2$) obtained between 21-28 d after administration.

Conclusions: Given the results, it can be inferred that Fipronil showed peak concentrations (2.5 days) earlier than Fluazuron (6 days), followed by a gradual decrease of both active ingredients until the last observation day (42 days post-treatment).

Therapeutics & Pharmacology

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Therapeutic and residual efficacy of a novel Fluazuron combination against Rhipicephalus (Boophilus) microplus in naturally infested cattle

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Objectives: Two experiments were conducted to evaluate the efficacy of a new formulation containing Fipronil (1.25 mg / kg) + Fluazuron (2.5mg / kg), administered once, via pour-on, against Rhipicephalus (Boophilus) microplus in naturally infested cattle.

Materials and Methods: The studies were conducted in the city of Aquidauana, Mato Grosso do Sul State, Brazil (Experiment 1) and in the city of São José do Rio Pardo, São Paulo State, Brazil (Experiment 2). In both experiments, 20 animals (Holstein and Simmental, respectively) were randomized into two groups of 10 animals each (T01: treated with Fipronil + Fluazuron; T02: Saline 0.9 % Control) based on the average R. (B.) microplus counts (females with 4.5 and 8.0 mm in length present on the left side of each animal) from the three consecutive counts pre-treatment (days -3, -2 and -1). The therapeutic and residual efficacy of such formulation were assessed on days 1, 3, 7, 14, 21, 28 and weekly until 56 days post-treatment (DPT).

Results: In Experiment 1, the combination of Fipronil + Fluazuron obtained efficacy higher than 95% from the 3rd to the 35th DPT (96.26%, 100.00%, 99.58% and 97.81% respectively on days 3, 7, 14, 21, 28 and 35 post-treatment). The efficacy of such formulation remained higher than 80% until 42 DPT (88.79%).

In Experiment 2, the combination of Fipronil + Fluazuron obtained efficacy higher than 95% from the 7th to the 35th DPT (96.32%, 96.40%, 97.22%, 97.08% and 96.28% respectively on 7, 14, 21, 28 and 35 DPT). In this experiment, the residual efficacy was still higher than 90% (93.32%) at 42 DPT. In both studies, the R. (B.) microplus counts on the Fipronil + Fluazuron treated animals were significantly lower ($p \leq 0.05$) than the control animals from the 3rd to the 56th DPT.

Conclusions: Based on the results obtained in these experiments, the new combination of Fipronil + Fluazuron administered once via pour-on, showed high efficacy against Rhipicephalus (Boophilus) microplus in naturally infested cattle.

Therapeutics & Pharmacology

P04-004-112

Therapeutic and residual efficacy of a novel combination of Fipronil and Fluazuron against Rhipicephalus microplus in artificially infested cattle

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Objectives: The experiment was conducted to evaluate the efficacy of a new formulation containing Fipronil (1.25 mg / kg) + Fluazuron (2.5mg / kg), administered once via pour-on against Rhipicephalus (Boophilus) microplus in artificially infested cattle.

Materials and Methods: The experiment was conducted in the city of Abadia dos Dourados, Minas Gerais State, Brazil. 28 crossbreed steers were randomized into four groups of seven animals each, based on the average counts of engorged female ticks detached from each animal on three consecutive days prior to treatment (days -3, -2 and -1): T01: Association of Fipronil (1.25 mg / kg) + Fluazuron (2.5 mg / kg); T02: Fipronil standalone (1.0 mg / kg); T03: Fluazuron standalone (2.5 mg / kg); T04: Saline 0.9% Control. The therapeutic and residual efficacy against R. (B) microplus was evaluated by daily counts of all female ticks detached from each animal, from the 1st to 60th days post treatment (DPT).

Results: In this study, T01 showed mean therapeutic efficacy (1 to 23 DPT) of 95.8% while T02 and T03 presented respectively 94.0% and 89.0 %. T01 presented efficacy ≥90% from the 4th to 45th DPT, with efficacy ≥95% in 30 counting days during this period. On the other hand, T02 and T03 presented, respectively, efficacy ≥90% from the 4th to 40th DPT and from the 5th to the 39th DPT (31 and 27 counting days with efficacy higher than 95%). The efficacy of such formulations were above 80% until 57 (T01), 42 (T02) and 48 (T03) DPT. The statistical comparison showed that T01 mean counts were significantly lower (p≤0.05) than the control group counts from the 3th DPT until the end of the study (60 DPT). The animals from T02 presented statistically lower mean counts compared to the control group (p≤0.05) from 2th to 46th DPT while this difference was significant for T03 treated animals from 4th to 56th DPT.

Conclusions: Based on the results from this experiment, the new combination of Fipronil + Fluazuron administered once via pour-on, showed higher mean therapeutic efficacy and longer residual protection against Rhipicephalus (Boophilus) microplus in naturally infested cattle when compared to standalone formulations of Fipronil and Fluazuron.

of dairy cattle in France. Getting new data for this topic is relevant because S. aureus is one of the most frequently isolated bacteria in milk samples from sub-clinical cases of bovine mastitis in France (about 18% excluding sterile or contaminated samples). Finally, these data may help reaching a better and responsible use of antibiotics and possibly contribute to the reduction of antibiotic use in France of 25% from 2012 to 2017.

Materials and Methods: The susceptibility of 80 isolates of S. aureus to antibiotics was assessed by the standard agar dilution method. These strains were isolated using milk samples from cows affected by clinical or sub-clinical mastitis. They were chosen at random from all milk samples received in 2013 by ISAE 35 laboratory from all over France and the strains tested were epidemiologically unrelated (eg not coming from the same episode of disease or the same herd - guideline EMEA/CVMP/627/01).

A MIC-determination was performed using the agar dilution method according to the standard method (standard CLSI VET01-A4). Twelve antimicrobial agents – penicillin G, cloxacillin, nafcillin, cephalexin, cefazolin, cephapirin, cephalonium, cefquinome, amoxicillin-clavulanic acid, tylosin, gentamicin, and enrofloxacin – were investigated because they are frequently used in the field. The results are expressed in µg/mL.

Results: All S. aureus isolates were sensitive to cloxacillin, nafcillin, cefazolin, cephapirin, cephalonium and amoxicillin-clavulanic acid. Antibiotic resistant strains were observed for penicillin G (n=17, 21%), cephalexin (n=2, 3%) and cefquinome (n=2, 3%). This percentage (21%) of S. aureus strains resistant towards penicillin G is lower than the 30% mentioned in the RESAPAPH report.

The antimicrobial susceptibility of S. aureus towards the different cephalosporins varies significantly. For example, the MIC₉₀ of cephalonium is only 0.125µg/mL towards S. aureus, when MIC₉₀ of cephalexin is 4µg/mL.

There is a high and homogeneous level of antimicrobial susceptibility of S. aureus towards gentamicin (all MIC ≤ 0.25µg/mL). But, the antimicrobial susceptibility of S. aureus towards tylosin is just below the breakpoint (MIC₉₀ = 2 µg/mL). A bimodal repartition of the MIC of S. aureus strains towards tylosin was seen, as previously described in a German study, with 2 strains (3%) showing a MIC of tylosin >256 µg/mL. S. aureus resistance towards penicillins G is mainly due to the presence of the blaZ gene, coding for a β-lactamase. The BlaZ gene is frequently (30%, RESAPAPH report) detected in the strains of S. aureus isolated in France. Because the difference between phenotypic resistance (MIC determination) and genetic resistance is mainly due to the inducible expression of this gene, it seems that resistance is only observed if bacteria have previously been in contact with a β-lactam.

Conclusions: This in vitro study shows that the resistance of S. aureus towards penicillin G is widely observed and the antimicrobial susceptibility of S. aureus towards the macrolides is not optimal (high MIC, and some strains are resistant towards this antibiotic group). Although in vitro MIC studies do not always predict in vivo treatment success, based on these MIC data alone, the preferred antibiotics for treatment of S. aureus are cephalonium, gentamycin, cephapirin, cloxacillin, nafcillin, cefazolin, amoxicillin-clavulanic acid.

Therapeutics & Pharmacology

P04-004-113

Antimicrobial susceptibility of Staphylococcus aureus isolated in bovine mastitis in France

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Objectives: To determine the minimal inhibitory concentrations (MIC) of antimicrobials for Staphylococcus aureus (S. aureus) from the milk

Therapeutics & Pharmacology

P04-004-114

Flunixin meglumine transdermal pour-on solution as adjunct therapy in the treatment of bovine respiratory disease in calves less than 8 weeks of ageSiddhartha Torres^{1,*} Julien Thiry¹ Vincent de Haas¹ Philippe Brianceau¹¹Merck Animal Health, Madison, United States

Objectives: Whether Bovine Respiratory Disease (BRD) causing factor is physical, environmental, or infectious, a sequence of events occurs resulting in inflammation. It is advisable that NSAID and anti-infective agents are used concurrently. Flunixin is a NSAID commonly used for the relief of pain and control of inflammation and pyrexia associated with diseases of different origin and nature. A novel 50 mg/mL flunixin transdermal (Finadyne® Transdermal) is available for cattle. The objective of the study was to demonstrate the safety and effectiveness of flunixin transdermal in the treatment of BRD in juvenile calves.

Materials and Methods: The study was conducted following the GCP guidelines. A total of 49 calves of less than 8 weeks of age, showing severe signs of respiratory disease, were randomly assigned to treatment with either the test product, flunixin transdermal, administered topically once, or the control product, carprofen, administered by injection once, on day 0. All animals received cefquinome on days 0 and 2. The animals were observed for clinical signs of disease for 6 hours post-treatment and daily for 5 consecutive days.

Results: The decrease in rectal temperature 6 hours post-treatment was greater in the flunixin group (-1.7°C) compared to the control group (-1°C). This difference was statistically significant (p<0.0001) and the superiority of the flunixin transdermal to the control was confirmed.

In the following days, rectal temperature and clinical index (depression and respiratory signs) improved similarly over time in both treatment groups.

Neither flunixin nor control had a negative influence on the health status including appetite and faecal consistency, confirming that both products are safe.

Conclusions: The new 50 mg/mL flunixin transdermal was shown to be safe and to have a strong anti-pyretic effect and anti-inflammatory properties, and makes it a very convenient and suitable adjunct therapy to anti-infective therapy used in cases of respiratory infections in juvenile calves less than 8 weeks of age.

Therapeutics & Pharmacology

P04-004-115

Effect of two different bovine somatotropins on milk yield of Brazilian dairy cowsHenderson Ayres^{1,*} Marcilio Nichi² Sandro L. Viechnieski³¹MSD Animal Health, ²Department of Animal Reproduction of USP, Sao Paulo, ³Star-Milk Farm, Céu Azul, Brazil

Objectives: Bovine somatotropin (bST) is recognized as a stimulus for increased milk production. The objective of this trial was to evaluate milk yield in a high-producing dairy cattle herd in response to two commercial sources of bovine somatotropin (bST) administered every 14 days

totalizing 16 applications. Our hypothesis was that the use of Boostin promotes greater milk production compared to Lactotropin.

Materials and Methods: Eighty Holstein cows from a commercial farm located in the south region of Brazil, averaging 1.76 ± 1.16 lactations, 77.1 ± 21.9 days in milk (DIM) and 37.5 ± 6.5 kg/d of milk were randomly assigned to one of 2 treatment groups using milk production during the 7-day pretreatment period as the blocking criterion. Treatments consisted of 16 consecutive subcutaneous injections of 500 mg of bST (Boostin, MSD Saúde Animal, Brazil) or 500 mg of bST (Lactotropin, Elanco Saúde Animal, Brazil), both administered at a 14-day interval. All cows were milked 3 times a day and received the same total mixed feed 5 times daily, consisting of corn silage, ryegrass silage, corn grain ground, soybean meal, whole cottonseed, soybean hulls, urea, minerals, and vitamins. Cows were housed in free stalls. Data were analyzed with the SAS procedure mixed with a model containing the continuous effect of the covariate and the fixed effects of block, treatment, day, number of administrations and the interaction between treatment and day and treatment and number of applications. Data were compared considering each cycle of administration for two procedures: milk yield and relative milk yield. Relative milk yield was defined as the increased milk yield from each day after administration of bST relative to the production of one day before each administration.

Results: The results show that mean square of cow nested within treatment was used as the error term to test the treatment effect. Boostin-treated cows yielded 1.78 kg/day (P = 0.02) or 1.05 times (P < 0.01) more milk than Lactotropin-treated cows; (milk yield – 39.08 ± 0.12 versus 37.30 ± 0.12 kg/d; relative milk yield – 1.09 ± 0.001 versus 1.04 ± 0.001 times, respectively). Treatment * day interaction was also an important source of variation (P < 0.01). Further, the curves pattern was similar in both graphs.

Conclusions: Our hypothesis proved to be correct. In this trial we observed statistical differences in the milk yield response when comparing both commercial sources of bST. Boostin-treated cows yielded significantly more milk than Lactotropin-treated cows.

Therapeutics & Pharmacology

P04-004-116

Efficacy of injectable florfenicol plus flunixin combination against an experimentally induced Mycoplasma bovis infection in calvesJulien Thiry¹ Sophie Rubion² Cliff Ramage³ David Reddick³ Vincent de Haas¹ Siddhartha Torres^{1,*}¹Merck Animal Health, Madison, United States, ²MSD Animal Health Innovation, Beaucauzé, France, ³Moredun Scientific Ltd, Penicuik, United Kingdom

Objectives: The objective of this blinded, randomised study was to demonstrate the efficacy of a combination of florfenicol and flunixin (Resflor®), against an experimental Mycoplasma bovis challenge in calves, in comparison to a negative control saline and a positive control tulathromycin associated with flunixin.

Materials and Methods: A total of 230 calves between 4 and 8 weeks of age, confirmed to be M. bovis antibody and antigen negative, were challenged daily for 3 consecutive days by intratracheal deposition of 12 mL pure M. bovis culture (5.14x10⁹ cfu, European isolate 208/B01). For 4 days after challenge, calves meeting enrolment criteria (pyrexia >39.5°C and abnormal respiration and/or depressed demeanour) were

enrolled for treatment with either saline (N=21), tulathromycin plus flunixin (N=64) or florfenicol-flunixin combination (N=64). After treatment, animals were observed daily for seven days for clinical recovery (rectal temperature, respiration and demeanour). On day 7 post-treatment, animals were euthanized, lungs collected for lesions scoring and lung lavage fluid collected for *M. bovis* bacteriology.

Results: After treatment, rectal temperature values, respiration and demeanour scores significantly decreased within 24 hours post-treatment with florfenicol-flunixin combination and tulathromycin plus flunixin, while they remained high for 7 days post-treatment with saline ($p < 0.0001$). At both day 4 and day 7 post-treatment, clinical recovery with florfenicol-flunixin combination was significantly superior to saline ($p < 0.0001$) and non-inferior to tulathromycin plus flunixin. The proportion of lungs with *M. bovis* lesions was 6.6% for florfenicol-flunixin combination, 5.25% for tulathromycin plus flunixin and 19.2% for saline.

Recovery of *M. bovis* from lung fluid was significantly lower for florfenicol-flunixin combination than for saline (2.27×10^5 cfu/mL versus 1.33×10^7 cfu/mL – $p = 0.0020$) and for tulathromycin plus flunixin (2.27×10^5 cfu/mL versus 2.53×10^6 cfu/mL – $p = 0.0120$).

A significant difference in daily weight gain over the 7-day study was observed in florfenicol-flunixin combination and tulathromycin plus flunixin (mean 0.40 kg/day – $p < 0.0001$), compared to saline (0.13 kg per day – $p = 1.0000$).

Conclusions: Data from this study showed that the administration of a combination of florfenicol and flunixin (Resflor®) provided an efficient clinical and microbiological cure of *Mycoplasma bovis* pneumonia in calves.

Therapeutics & Pharmacology

P04-004-117

Biochemical and blood gas alterations in buffalo whole blood stored in CPDA-1 and CPD/SAG-M bags.

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Objectives: The whole blood transfusion in farm animal's objective the restoration of the capacity of transport and diffusion of oxygen to the tissues in cases of significant reduction in hemoglobin, as in severe anemia or acute blood loss. Thus, it was aimed to evaluate the biochemical and blood gas analysis of buffalo whole blood stored in CPDA-1 and CPD/SAG-M bags for 42 days.

Materials and Methods: We used ten adult, male, healthy, mixed-breed buffalo, from each 900 g of whole blood were collected and subsequently stored in CPDA-1, and CPD/SAG-M bags (450 g in each bag) and kept under refrigeration at 2°C to 6°C for 42 days. Whole blood stored in plastic bags were evaluated at seven moments: D0 (immediately after collection of blood), D7 (seven days after collection), D14 (fourteen days of collection), D21 (21 days after collection), D28 (28 days of collection), D35 (35 days of collection) and D42 (42 days after collection). At those moments blood gas, biochemical (glucose, total protein, albumin, sodium, potassium, cholesterol and lactate) and microbiological parameters were

analyzed. Two-way repeated measures ANOVA was used to compare the effect of time and type of blood bag.

Results: In both blood bags, increases ($p < 0,05$) were observed in oxygen pressure (pO_2), carbon dioxide pressure (pCO_2), lactate and potassium during the storage period. The reductions ($p < 0,05$) were noticeable in the concentration of sodium, bicarbonate, glucose and the pH. Cholesterol, total protein and albumin remained unaltered. The microbiological analysis reveal contamination in two bags from the same animal, which were removed from the study. The remained 18 blood bags from nine buffalos did not present any contamination until the end of the study. The buffalo whole blood stored in CPDA-1 and CPD/SAG-M plastic bags undergoes biochemical and blood gas alterations during the storage, however it remained viable for transfusion when stored for up to 42 days at temperatures of 2 to 6 °C.

Conclusions: In general, there were no clinical importance differences in the conservation of whole blood buffalo between the two types of bags (CPDA-1 and CPD/SAG-M) evaluated, being both suitable for use in this species. Whole blood of buffalo kept refrigerated for 42 days in plastic bags did not undergo biochemical and blood gas changes that could prevent its use for blood transfusion.

Therapeutics & Pharmacology

P04-004-118

Acute Phase Proteins as Predictive Markers to Diarrhea and Evaluation of Treatment with Oral Electrolyte and Energy Replacement

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Objectives: The aim of the study was to evaluate the acute phase proteins levels (haptoglobin and paraoxonase) in calves as predictive markers to diarrhea occurrence and to evaluate the treatment with oral electrolyte and energy replacement.

Materials and Methods: This study was conducted in a commercial farm located in southern Rio Grande do Sul - RS - Brazil. Were used 21 Holstein calves, monitored from birth to sixth week of life. During the experimental period were performed clinical tests twice weekly on all animals. After showing diarrhea clinical signs, the calves were randomly assigned to the four groups: Healthy animals without diarrhea (Healthy; n = 6), Group Antibiotic (ATB; n = 4), Group Antibiotic + Intravenous support (ATB + IS; n = 4) and Group Antibiotic + Oral support (ATB + OS; n = 7). The active ingredient used in the groups that received antibiotics was the fast action enrofloxacin (Kinetomax®, Bayer Animal Health, Germany), a single dose of 7.5 mg/kg of body weight (BW), intramuscularly (IM). The support used in the treatment ATB + IS consisted of oral administration of activated charcoal (6 g) once daily for 3 days, flunixin meglumine (Flunamine®, Bayer Animal Health, Germany) at a dose of 1,1 mg/kg of body weight, intramuscularly, and in cases of dehydration, intravenous (IV) fluid NaCl 0.9% based on the volume to be established according to the degree of dehydration. For ATB + OS group has been replaced the IV fluid by supplementation with oral replacement solution (Glutellac®, Bayer Animal Health, Germany) at a dose of 50 ml, added to milk, twice a day. Were collected blood samples every seven days on all animals. After the diarrhea diagnostic was collected blood sampling at 0, 24, 72

and 120 h in relation to the diagnosis, to acute phase proteins evaluation (paraoxonase and haptoglobin).

Results: In the period before the disease, paraoxonase levels were lower in calves that had diarrhea (15.55 ± 1.26 U/mL) compared to healthy calves (67.29 ± 2.19 U/mL; $P < 0.0001$), and already at day -28 groups have differed as to the paraoxonase levels ($P < 0.05$). Similarly the haptoglobin levels differ between groups ($P = 0.04$), with higher values of haptoglobin in healthy animals (1.23 ± 0.08) than in animals that developed diarrhea (1.23 ± 0.14). During the 120 hours of evaluation after diagnosis of diarrhea, all groups submitted to treatment differed from healthy animals ($P < 0.0001$) in the levels of paraoxonase (Healthy: 78.50 ± 2.32 U/mL; ATB: 10.64 ± 2.84 U/mL; ATB + IS: 11.51 ± 2.84 U/mL; ATB + OS: 16.90 ± 2.15 U/mL). When comparing the treated groups, the ATB group tend to have lower values of paraoxonase in relation to ATB + OS group ($P = 0.08$). Regarding the haptoglobin levels during diarrhea there was a difference trend between groups (Healthy: 3.02 ± 0.64 ; ATB: 4.16 ± 0.78 ; ATB + IS: 4.66 ± 0.78 ; ATB + OS: 2.24 ± 0.59 ; $P = 0.06$), and ATB + OS group showed lower levels of haptoglobin than ATB ($P = 0.05$) and ATB + IS groups ($P = 0.02$).

Conclusions: Thus, the paraoxonase proved to be an important marker to predict the occurrence of diarrhea in calves, while haptoglobin need for further studies to define whether it is a good marker for this. Regarding the treatments for diarrhea, antibiotic treatment associated energy and electrolyte replacement orally showed best results in terms of inflammatory profile, indicating a lesser severity of the disease.

Therapeutics & Pharmacology

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Pharmacokinetics and distribution in interstitial and pulmonary epithelial lining fluid of danofloxacin in ruminant and preruminant calves

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Objectives: The objective of this study was compare active drug concentrations in the plasma versus different effector compartments including interstitial fluid (ISF) and pulmonary epithelial lining fluid (PELF) of healthy pre-ruminating (3-week old) and ruminating (6-month old) calves.

Materials and Methods: Eight unweaned Holstein calves, 2-3 weeks of age, weighing between 41-53 kg were classified as preruminants. Eight weaned calves, 6 months of age and weighing between 151-214 kg at time of study were classified as ruminating calves. Approximately 24 h prior to start of the study, calves were restrained for placement of an intravenous catheter and a subcutaneous tissue probe. A single subcutaneous (s.c) injection of danofloxacin (8 mg/kg) (Advocin™; Zoetis) was administered to each calf in the neck per label instructions. Blood samples were taken from the jugular vein and transferred to heparinized tubes at 0 (pretreatment), 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72 and 96 hours post administration and stored at -80°C until analysis. Interstitial fluid (ISF) was collected at 0 (pretreatment), 2, 4, 8, 10, 12, 24, 48, 72 and 96 h after s.c. administration. In order to determine drug concentrations in pulmonary epithelial lining fluid, multiple bronchoalveolar lavages (BAL) were performed at 2, 12 and 24 h after administration. Sterile saline (100 mL) was infused into the lungs.

Plasma, ISF and BAL fluid were analyzed by high-performance liquid chromatography (HPLC) with fluorescence detection. Estimation of the amount of PELF sampled by BAL fluid was performed using the urea dilution method. Plasma protein binding analysis was performed using ultracentrifugation. Danofloxacin concentrations were evaluated using compartmental and non-compartmental analysis.

Results: After s.c. administration, the maximum concentration in plasma (C_{max}) reached 1.7 $\mu\text{g/mL}$ for preruminant and 1.6 $\mu\text{g/mL}$ in ruminant calves and occurred at 3.1 and 1.4 h post administration respectively (T_{max}). The absorption rate constant differed significantly between the two age groups. The terminal elimination ($t_{1/2}$) was estimated as 20.36 hrs for preruminant calves and 15.31 hrs for ruminant calves. These differences were not statistically significantly different. When considered the slope from T_{max} to hour 24 rather than the terminal elimination phase, the $t_{1/2}$ estimates were ranged from 4.23 to 10.16 hrs in the preruminant calves and 4.0 to 5.44 hrs in the ruminant calves. T_{max} for ISF fluid occurred later than plasma in both groups. The area-under-curve for plasma from 0 to 96 hours (AUC_{0-96}) was not statistically significant between the two groups. Whether considering the ISF to total or free plasma danofloxacin concentrations, the ISF/Plasma concentration ratios tended to be higher in ruminant calves as compared to that seen in the preruminant calves. The maximum concentration in PELF was noted to occur at 2 hours post administration in both preruminant and ruminant calves, with estimated PELF concentrations far exceeding the concentrations seen in plasma and ISF. Greater variability in drug concentrations in PELF were seen in the preruminant calves as compared to ruminating calves. The average protein binding for danofloxacin in 3 week and 6 month old calves decreased over a linear range of 0.01 to 10 $\mu\text{g/mL}$ and averaged $32 \pm 25\%$ and $37 \pm 16\%$ respectively.

Conclusions: This study reported the pharmacokinetics of subcutaneous danofloxacin when used at the approved adult cattle dose (8 mg/kg) in 2 age groups of calves. Results demonstrate two fundamental differences included the rate of drug absorption and CL/F. Trends towards differences in V_d/F and $t_{1/2}$ were also observed. A deep or third compartment, which has not been identified in previous studies, was detected and was most evident in the ruminating calves. This contributed to age-associated differences between the relationships of ISF/plasma over time. Further studies are warranted to compare drug concentrations diseased with healthy calves.

Therapeutics & Pharmacology

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A Study To Examine The Relationship Between Uterine Pathology And Depletion Of Oxytetracycline In Plasma And Milk After Intrauterine Infusion.

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Objectives: The objective of this study was to use highly sensitive laboratory technology to determine the plasma and milk concentrations of oxytetracycline following intrauterine infusion in post-partum dairy cows. Additionally, we hoped to determine if the variability in distribution

and elimination of oxytetracycline from the uterus to plasma and milk was associated with the degree of uterine pathology between cases of metritis as determined by uterine severity scores. Our hypothesis is that cows experiencing more severe uterine pathology will have higher plasma and milk oxytetracycline concentrations compared to less severely affected cows.

Materials and Methods: Thirty-two cows received a single treatment of 4 g of oxytetracycline via intrauterine infusion. Blood and milk samples were collected prior to intrauterine therapy and throughout the trial period of 96 h post infusion. Uterine severity scores were assigned at initiation of therapy and every 24 h throughout the remainder of the trial. Plasma and milk samples were analyzed for oxytetracycline concentrations using liquid chromatography coupled with mass spectrometry. Drug concentrations in plasma and milk were modeled using non-compartmental pharmacokinetic analysis. Maximum concentrations and time at maximum concentration for plasma and milk were calculated directly from the raw data. Area under the concentration curve was calculated using the linear trapezoidal rule with linear interpolation.

Results: Following treatment, oxytetracycline rapidly diffused to plasma and milk. The mean (\pm SEM) Cmax of oxytetracycline in plasma was 220.6 ± 31.0 ng/mL was recorded at a Tmax of 23.2 ± 1.3 h after intrauterine infusion. The mean (\pm SEM) plasma area under the curve (AUC) was 8744.8 ± 1335.6 h \times ng/mL. The mean (\pm SEM) Cmax of oxytetracycline in milk was 136.2 ± 19.1 ng/mL was recorded at a Tmax of 22.0 ± 1.8 h after intrauterine infusion. The mean (\pm SEM) milk AUC was 5962.9 ± 945.6 h \times ng/mL. Eighteen of the cows still had detectable levels of oxytetracycline in milk 4 days after intrauterine infusion. The mean (\pm SEM) milk concentration of oxytetracycline in plasma at the end of the study was 13.3 ± 4.0 ng/mL (range 0 - 102.7 ng/mL). Greater uterine pathology score at the initiation of treatment showed a significant positive correlation with higher milk oxytetracycline concentration at the second milking following treatment ($R^2 = 0.46$; $P = 0.01$), but there was no correlation between initial uterine pathology score and oxytetracycline concentration at the conclusion of the study ($R^2 = -0.06$; $P = 0.75$).

Conclusions: Intrauterine administration of oxytetracycline is considered to be an extra-label therapy in the United States. When prescribing extra-label therapy, the veterinarian is primarily responsible for specification of a withdrawal time for milk and meat to ensure the products are below established legal tolerances or target test levels. Applying the established milk withhold and published sensitivity levels of the available bulk tank residue detection kits to the milk concentrations of study cows would indicate that most cows should test negative by 96 h after oxytetracycline treatment.

Therapeutics & Pharmacology

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Effectiveness of pegbovigrastim against naturally occurring clinical mastitis in periparturient cows and heifers in a multi-site European field study

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Objectives: The objective of this clinical field study was to evaluate the effectiveness of pegbovigrastim against naturally occurring mastitis in periparturient cows and heifers. This clinical efficacy study was

conducted at 35 commercial farms considered to be representative of European dairy husbandry in four countries, Hungary, Germany, Netherlands and the United Kingdom.

Materials and Methods: A total of 2561 clinically healthy periparturient dairy cows and heifers (typical European dairy breeds) were enrolled in the study after physical examination. Approximately 7 days before the anticipated date of parturition, animals received subcutaneous injections of either sterile saline (0.9% NaCl) solution (control treatment) or pegbovigrastim (contents of one syringe). Within 24 hours after parturition, each cow received a second syringe of the assigned treatment (day 0 treatment). Cows were observed at each milking for clinical signs of mastitis for the first 30 days of lactation. Clinical cases of mastitis were identified by farm or study staff at the time of milking based on abnormal appearance of the quarter (swelling, heat, pain) and/or changes in the appearance of milk (clots or flakes, watery appearance, discoloration), with or without systemic signs of disease (fever, loss of appetite).

Results: The combined multi-site study enrolled cows or heifers from 5 commercial farms in the United Kingdom, 12 in Germany, 5 in Hungary and 12 in the Netherlands. The multi-site analysis demonstrated a statistically significant ($p=0.0095$) reduction of 26% in the incidence of naturally occurring clinical mastitis in the pegbovigrastim treated group relative to the control group. From the 1230 cows enrolled in the Control group, 156 cows showed clinical signs of mastitis (13%) and in the pegbovigrastim group 1235 were enrolled and 116 cows showed signs of clinical mastitis (9%).

Conclusions: Results of this multi-site field study demonstrate pegbovigrastim is effective as an aid in a mastitis management programme, to reduce the risk of clinical mastitis in periparturient dairy cows and heifers during the first 30 days of lactation. Pegbovigrastim provides a novel approach for restoring periparturient neutrophil function to normal levels enabling a reduction in the incidence of new intramammary infections during lactation. Ultimately less cows impacted by clinical mastitis equates to less milk loss, less antimicrobial use by the dairy sector, fewer cull cows, improved animal welfare and financial benefits for the farmer.

Therapeutics & Pharmacology

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Early postpartum treatment of commercial dairy cows with nonsteroidal antiinflammatory drugs increases whole-lactation milk yield

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Objectives: Previous research has shown that postpartum administration of the nonsteroidal antiinflammatory drug (NSAID) sodium salicylate can increase 305-d milk yield in older dairy cattle (parity 3 and greater). However, in this prior work, sodium salicylate was delivered to cows via the drinking water, a method that does not align well with current grouping strategies on commercial dairy farms. The objective of the current study was to replicate these results on a commercial dairy farm

with a simplified treatment protocol and to compare sodium salicylate with another NSAID, meloxicam.

Materials and Methods: Dairy cattle in their second lactation and greater ($n = 51/\text{treatment}$) were alternately assigned to 1 of 3 treatments at parturition, with treatments lasting for 3 d. Experimental treatments began 12 to 36 h after parturition and were (1) 1 placebo bolus on the first day and 3 consecutive daily drenches of sodium salicylate (125 g/cow per day; SAL); (2) 1 bolus of meloxicam (675 mg/cow) and 3 drenches of an equal volume of water (MEL); or (3) 1 placebo bolus and 3 drenches of water (CON). Blood samples were collected on the first day of treatment, immediately following the last day of treatment, and 7 d after the last day of treatment; plasma was analyzed for glucose, β -hydroxybutyrate (BHB), free fatty acids, haptoglobin, and paraoxonase. Milk production, body condition score, reproductive status, and retention in the herd were monitored for 365 d posttreatment, and effects of treatment, parity, days in milk, and interactions were evaluated in mixed effects models. Significance was declared at $P < 0.05$.

Results: Whole-lactation milk and protein yields were greater in NSAID-treated cows, although 305-d fat production was not affected. Both MEL and SAL increased daily milk production compared with CON ($P < 0.05$; 36.8, 36.3, and 32.8 \pm 2.2 kg/d, respectively). Analysis of 305-d mature-equivalent milk yield resulted in similar findings, with MEL and SAL increasing yields compared with CON (both $P < 0.03$; 11,205, 11,411, and 10,472 \pm 486 kg, respectively). We found no evidence of treatment interactions with time ($P = 0.56$), although the contrasts between NSAID treatments and CON did not become significant until 7 wk in milk. There was a significant interaction of treatment and parity for plasma glucose concentration; MEL increased plasma glucose concentrations compared with CON and SAL in older cows. Sodium salicylate decreased plasma BHB concentration compared with MEL at 7 d posttreatment, although no difference was detected immediately following treatment. Haptoglobin concentrations were elevated in SAL cows compared with CON. There was a tendency for CON cows to be removed from the herd more quickly than MEL cows (42 vs. 26% at 365 d posttreatment ($P = 0.06$)). Body condition score, concentrations of plasma free fatty acids and paraoxonase, and time to pregnancy were not affected by treatment.

Conclusions: Early-lactation treatment with NSAIDs from 2 different classes increased whole-lactation milk yield by 7 to 9%, with only a 3-d treatment window immediately post-partum. When possible, NSAID effects should be recorded throughout the entire lactation, as treatment differences may be delayed and not immediately apparent following administration, such as in the current experiment. Furthermore, the tendency for MEL to delay the mean time to removal from the herd points to a fruitful area of investigation for future research.

Therapeutics & Pharmacology

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Antibiotic susceptibility of non-viral pathogens isolated from calves with BRD.

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Objectives: If Bovine Respiratory Disease (BRD) occurs, treatment of non-viral pathogens (*Mannheimia haemolytica* (*M. haemolytica*), *Pasteurella multocida* (*P. multocida*), *Mycoplasma bovis* (*M. bovis*) and *Histophilus somni* (*H. somni*)) should be effective to control damage caused by this disease. To ensure the efficacy of BRD treatment during

outbreaks, both the presence and antibiotic sensitivity of pathogenic bacteria should be determined. The objective of the study was to isolate BRD causing bacteria from veal and dairy calves during disease outbreaks and to determine their antibiotic sensitivity.

Materials and Methods: At the start of BRD outbreaks on 178 Dutch farms between March 2013 and October 2015, deep nasopharyngeal swabs (2/3 from veal calves, 1/3 from dairy calves) were taken. Calves with BRD symptoms and before treated with antibiotics were enrolled in the study. The inclusion criteria for BRD symptoms were temperature, coughing, nasal and eye discharge and position of the ears. Each symptom was scored on a scale from 0 till 3 and calves should score at least 5 points. 1103 calves were enrolled and sampled for bacteriological culture and antibiotic susceptibility testing. The samples of each outbreak were inoculated directly on blood agar and chocolate agar plates and for mycoplasma inoculation on preheated PAM and PAM+ plates. The plates were incubated under aerobic and micro aerophilic conditions. The inhibition zones of these BRD bacteria were determined using sensitivity discs (paper discs) containing doxycycline/tetracycline, erythromycin/tylosin/tilmicosin, florfenicol, trimethoprim sulfa, amoxicillin/ampicillin/penicillin, flumequin and enrofloxacin/marbofloxacin and read according to the EUCAST tables. For *M. bovis* no susceptibility testing was done, because the used method is not suitable for susceptibility testing of *M. bovis*. The choice of these antibiotic sensitivity discs is based on the Dutch formulary for prudent use of antibiotics. In the Dutch formulary for prudent use of antibiotics the classification of the groups (first, second and third choice) is based on working spectra and the effects on the selection pressure of antibiotic resistance.

Results: *M. haemolytica* was isolated in 297 samples in 104 BRD outbreaks, *M. bovis* in 403 samples in 125 BRD outbreaks, *P. multocida* in 572 samples in 159 outbreaks and *H. somni* in 2 samples in 2 outbreaks. Percentage of farms where the pathogen was isolated was 58% for *M. haemolytica*, 70% for *M. bovis*, 89% for *P. multocida* and 1% for *H. somni*. Percentage of samples, where *M. haemolytica* was isolated ($n = 104$), sensitive for an antibiotic was 12% for doxycycline/tetracycline, 70% for erythromycin/tylosin/tilmicosin, 95% for florfenicol, 80% for trimethoprim sulfa, 60% for amoxicillin/ampicillin/penicillin, 87% for flumequin and 90% for enrofloxacin/marbofloxacin. Percentage of samples, where *P. multocida* was isolated ($n = 159$), sensitive for an antibiotic was 26% for doxycycline/tetracycline, 53% for erythromycin/tylosin/tilmicosin, 98% for florfenicol, 50% for trimethoprim sulfa, 90% for amoxicillin/ampicillin/penicillin, 92% for flumequin and 95% for enrofloxacin/marbofloxacin. *H. somni* ($n = 2$) demonstrated to be 100% sensitive to all antibiotics tested.

Conclusions: Compared to previous antibiotic resistance data of *M. haemolytica* and *P. multocida*, this study shows an increase of antibiotic resistance. Antibiotic susceptibility for *M. haemolytica* and *P. multocida* showed low sensitivity for first choice antibiotics (doxycycline/tetracycline, erythromycin/tylosin/tilmicosin, and trimethoprim sulfa), but high susceptibility towards florfenicol. The increasing antibiotic resistance found in this study towards first choice antibiotics, suggests more emphasis should be put on the prevention of BRD. However, if BRD occurs, florfenicol should be considered as a first choice treatment.

Therapeutics & Pharmacology

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Efficacy, effectiveness and efficiency of an alternative treatment protocol of bovine respiratory disease with reduced amount of antimicrobials

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Objectives: The context of prudent antibiotics use is a driver for new approaches. Considering the increased efficacy of some antibiotics on low bacterial inocula, dosage regimen could potentially be decreased when given at early stage of infection. We compared the standard regimen of marbofloxacin, a fluoroquinolone used to treat bovine respiratory disease (BRD), with a new protocol administering a fivefold reduction of the standard dose at an early stage of illness. Comparisons were made for (a) efficacy in calves experimentally infected with *Mannheimia haemolytica*, (b) effectiveness and efficiency in young bulls (YB) experiencing spontaneous pneumonia

Materials and Methods: To assess efficacy, 32 calves were intratracheally challenged with 4.10⁷ CFU of *M. haemolytica* A1. Based on an increase of their body core temperature continuously monitored with ruminal boluses, calves received either: (i) saline; (ii) 2mg/kg of marbofloxacin given 3h post challenge; or (iii) 2 or (iv) 10 mg/kg of marbofloxacin given 36h post challenge. Time course of bacterial burden at the infection site was assessed by PCR quantification on bronchoalveolar lavages. Clinical signs were daily scored during 5 days.

Thereafter, 195 YBs were randomly assigned to one of two experiment groups E2 and L10, defined by detection time of BRD (Early or Late) and marbofloxacin dosage regimen (2 or 10 mg/kg), and clinically surveyed during the first month on feed. As for calves, YBs' ruminal temperature was continuously monitored. In the E2 group, YBs with a ruminal temperature ≥40.2°C for more than 12h and presenting no or only slight signs of BRD were considered as Early detected and treated with 2 mg/kg of marbofloxacin. In the L10 group, YBs with moderate or severe clinical signs of BRD were considered as Late detected and treated with 10 mg/kg of marbofloxacin. If needed, a relapse treatment was administered. Clinical response to 1st line treatment and relapse treatment were recorded. The treatments units [amount (mg)/ Used Daily Dose (mg/kg)] for a standard YB of 300 kg at risk were calculated and summed up to determine total amount of antibiotics (TA).

Results:

Efficacy: did it work after experimental infection?

Calves in the three treated groups had lower bacterial counts within 24h after treatment and at necropsy, compared to the control group. An early treatment with 2 mg/kg of marbofloxacin lead to the elimination of *M. haemolytica* load. When treated with a 2 mg/kg of marbofloxacin at a later time, 50% of calves exhibited bacteria in their lungs. A higher regimen of 10 mg/kg allowed to obtain bacterial elimination in almost all the treated calves (85%). Animals in the 3 treated groups showed a greater clinical improvement within 24h, whatever the treatment compared to control group. At necropsy, gross lesions were rare in calves from treated groups, and moderate in calves from control group.

Effectiveness: did it work in field practice?

On YBs, 1st line treatment and relapse rates were higher in E2 (45 and 25%) vs L10 (37 and 7%). 97% and 100% of YBs treated in E2 and in L10 group were cured at the end of the study.

Efficiency: did it contribute to a more efficient use of resource in field practice?

TA was decreased by 33% and marbofloxacin amount by 63% in E2 vs L10 group. A standard YB received 173 and 471 marbofloxacin units and 54 and 9 relapse treatment units in E2 and L10 groups respectively.

Conclusions: Combination of early detection of disease with low antibiotic regimen improved efficiency of drug consumption at the herd level without affecting overall treatment effectiveness, compared to antibiotic administration at a later stage when bacterial burden is increased. Such findings evidence the feasible rationalization of antimicrobial use under field conditions, to limit its impact on public health.

Therapeutics & Pharmacology

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Novel anti-inflammatory properties of anti-microbials: Direct induction of leukocyte apoptosis and alterations of immune mediators by tulathromycin

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Objectives: Using live models of calves with pneumonia and bovine cell systems, these studies aimed at identifying novel mechanisms conferring anti-inflammatory benefits to anti-microbial compounds. The effects of tulathromycin were assessed in models where pneumonia was induced by live *Mannheimia haemolytica* or by zymosan particles, in order to distinguish direct anti-inflammatory effects from those associated with anti-microbial effects.

Materials and Methods: In keeping with the guidelines for ethical use of animals, Calves were challenged intra-tracheally with *Mannheimia haemolytica* or pro-inflammatory zymosan, and treated with vehicle or tulathromycin. Bronchoalveolar lavages were collected at 3 and 24 h post-challenge for analysis. Effects of tulathromycin were also investigated in circulating neutrophils and monocyte-derived macrophages. Data were obtained using reverse-phase High Performance Liquid Chromatography, transmission electron or fluorescent microscopy, quantitative Polymerase Chain Reaction, ELISA, and Western blotting. Results were compared using ANOVA and Tukey's test, or for data from studies in vivo, non parametric Kruskal-Wallis test.

Results: Tulathromycin reduced inflammation in the lungs, as demonstrated by post-mortem assessment of pathology and histology. Tulathromycin significantly increased (P<0.05) neutrophil apoptosis and efferocytosis (phagocytic elimination) by alveolar macrophages, and reduced pulmonary LTB₄, both in the lungs infected with live bacteria, or when inflamed with zymosan. These phenomena are known to be potent contributors to the resolution of inflammation. As the effects were reproduced in a model of sterile pneumonia induced by zymosan, the properties are independent of the anti-microbial effects of the compound. Caspase-dependent apoptosis of neutrophils and macrophages induced by tulathromycin was reproduced in vitro. We also found that tulathromycin directly reduces (P<0.05) the expression of pro-inflammatory CXCL8 (mRNA and protein) by bacterial lipopolysaccharide -stimulated neutrophils and macrophages, by inhibiting the transcription factor Nuclear Factor kappa B. These effects were independent of caspase activation, which indicates that they are separate from the pro-apoptotic benefits induced by the antibiotic. Inhibition (P<0.05) of pro-inflammatory Leukotriene B₄ synthesis in neutrophils induced by tulathromycin was caused by blockage (P<0.05) of pro-inflammatory phospholipases

(PLD and PLA₂). In contrast, Tulathromycin directly increases ($P < 0.05$) the synthesis of pro-resolution Lipoxin A₄, in resting and in Calcium²⁺ ionophore-activated neutrophils.

Conclusions: The findings illustrate novel mechanisms through which an anti-microbial compound such as tulathromycin may inhibit pro-inflammatory events and promote resolution of inflammation in the infected lung. The effects include induction of: 1) pro-resolution processes due to induction of leukocyte apoptosis, 2) direct immunomodulating effects (inhibition of pro-inflammatory Nuclear Factor kappa B and CXCL8, as well as Leukotriene B₄), and 3) the synthesis of Lipoxin A₄, a lipid mediator with potent pro-resolution properties. The findings may help lead to the targeted development of new antimicrobials with enhanced clinical benefits.

Comments: Bovine respiratory disease, such as that caused by Mannheimia haemolytica, is characterized by the exacerbated accumulation of neutrophils and pro-inflammatory mediators such as interleukin-8 (CXCL8) and Leukotriene B₄. This results in the severe inflammation ultimately responsible for pulmonary failure and death. Neutrophil cell death by apoptosis, rather than necrosis, helps resolve inflammation. Phagocytic clearance of apoptotic neutrophils by macrophages, known as efferocytosis, plays a key role in the resolution of inflammation. Hence, accumulation of neutrophils, leukotriene B₄, and CXCL8 are markers of inflammation-induced pathology, while lipoxin A₄ synthesis and neutrophil apoptosis mediate the resolution of inflammation. We postulated that the efficacy of tulathromycin in the treatment of bovine pneumonia could not be explained solely on the basis of its anti-microbial activity. The development of novel, clinically more effective anti-microbials is sorely needed. The discovery that an antimicrobial with superior clinical efficacy may confer strong and direct anti-inflammatory benefits by combining induction neutrophil apoptosis and inhibition of the local synthesis of pro-inflammatory cytokines and lipid mediators, while inducing the release of pro-resolution mediators, paves the way towards novel approaches in drug design.

Therapeutics & Pharmacology

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Efficacy of pegbovigrastim for reducing the incidence of naturally occurring clinical mastitis in periparturient dairy cows and heifers

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Objectives: Periparturient dairy cows and heifers are known to experience impaired neutrophil function leading to increased susceptibility to intramammary infections during early lactation. Bovine granulocyte colony stimulating factor (bG-CSF) has been shown to improve neutrophil function in periparturient cows. The objective of this study was to evaluate the effects of various dose levels of pegbovigrastim (PEGylated bG-CSF) upon the incidence of naturally occurring clinical mastitis in periparturient dairy cattle.

Materials and Methods: A total of 211 periparturient Hostein-Friesian cows and heifers were used for this study. Approximately seven days before their anticipated calving date, healthy animals were treated with either sterile saline or 5, 10 or 20 ug/kg of pegbovigrastim by subcutaneous injection. Animals were commingled and housed in a pen with dirt flooring which was kept wet to maximize exposure to bacterial pathogens and the incidence of naturally occurring clinical

mastitis. Within 24 hours of calving, each animal was dosed again with their assigned treatment. Absolute neutrophil counts (ANC) were determined for each animal prior to treatment, 24 hours after the first dose and on Day 7 post-calving. The condition of their mammary glands and milk were visually scored at each milking for 28 days after calving for abnormalities. Milk samples were aseptically collected from quarters exhibiting either an abnormal appearance or abnormal milk to identify pathogens. Daily milk production was recorded and milk composition was also assessed on Days 3, 5, 7 and 10 post-calving.

Results: All three doses of pegbovigrastim induced a statistically significant ($p < 0.01$) increase in ANC within 24 hours after dosing. Animals treated with either 20 or 10 ug/kg of pegbovigrastim exhibited ANC that were significantly ($p < 0.01$) increased relative to the saline controls on Day 7. Animals treated with 20 or 10 ug/kg of pegbovigrastim exhibited statistically significant ($p < 0.05$) reductions (72 or 50%, respectively) in the incidence of clinical mastitis relative to the saline controls. Animals treated with 5 ug/kg of pegbovigrastim exhibited a numerical reduction in the incidence of clinical mastitis which was not statistically significant ($p > 0.05$). The primary bacteria associated with these infections were environmental streptococci, coagulase negative staphylococci and coliforms. Administration of pegbovigrastim had no significant impact upon daily milk production or milk composition.

Conclusions: These results suggest pegbovigrastim is effective for reducing the incidence of naturally occurring clinical mastitis in periparturient dairy cows and heifers and warrants further investigation as a potential preventative intervention.

Therapeutics & Pharmacology

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Pharmacokinetics of Ampicillin-Sulbactam in Serum and Synovial Fluid Following Regional Intravenous Administration in the Distal Hind Limb of Cattle

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Objectives: To document the concentration versus time curve of ampicillin and sulbactam in synovial fluid (SYN) and serum of the digital circulation and central venous circulation following administration as a regional intravenous perfusion (RIVP) in the distal hind limb in cattle.

Materials and Methods: A prospective observational pharmacokinetic study was performed using six systemically healthy, non-pregnant, non-lactating adult Jersey or Jersey-cross cows. An RIVP was performed using a tourniquet applied to the right proximal metatarsus and 1.5 g combined ampicillin-sulbactam (1 g ampicillin, 0.5 g sulbactam) was administered in the right hind dorsal common digital vein (DCDV).

Indwelling catheters were used to collect serum (right hind DCDV and jugular vein) and synovial fluid (SYN; right metatarsophalangeal joint) at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 18, and 24 h post infusion. Furthermore, a single serum sample was collected from the abaxial proper plantar vein of the lateral digit of the right hind limb at 0.25 h post infusion. Serum and SYN were analyzed with high-performance liquid phase chromatography.

Results: Maximum mean concentrations of ampicillin in SYN, DCDV, abaxial proper plantar and jugular venous serum was 1995 (± 1011), 4827

(\pm 1883), 5423 (\pm 1953) and 2.5 (\pm 1.6) $\mu\text{g}/\text{mL}$ respectively. Sulbactam concentrations followed similar trends to ampicillin, and remained near or above the manufacturer's formulation of 2:1 ampicillin:sulbactam ratio. The concentration of ampicillin in SYN remained above the Clinical Laboratory Standards Institute breakpoints of 8 and 0.5 $\mu\text{g}/\text{mL}$ for 18.9 (range 15.4-24.9) h and 30.6 (range 23.4-38.4) h respectively. No adverse events were encountered.

Conclusions: Administration of 1.5 g combined ampicillin-sulbactam as an RIVP in the distal hind limb of cattle provided high concentrations of both compounds in

SYN for the majority of a 24 h interval, whereas systemic concentrations remained low. Therefore, the use of this drug combination as an RIVP may be clinically useful for treatment of distal limb infections in cattle.

Comments: Dr. Depenbrock's current affiliation is: Department of Production Animal Health, University of Calgary, Faculty of Veterinary Medicine, Calgary, Canada.

Toxicology

P04-004-199

Determination and identification of toxigenic fungi and their producing mycotoxins in cattle feed from subsistence and commercial farms in South

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Objectives: Exposure of animals to mycotoxins at high doses over a short period or low doses over an extended period, may affect their production, reproduction or growth. In addition, contaminated animal products (milk, meat and eggs) can possibly end up being consumed by both rural and urban populations and thus cause health effects with time, if exposure is prolonged. In this study, animal feed samples obtained from subsistence (SFs) and commercial farms (CFs) in selected areas of South Africa were analysed for fungal and mycotoxin contamination.

Materials and Methods: Feed samples (143) were obtained from rural subsistence farms (RSFs) (68) (mostly cereal remnants such as maize and brewer's meal) and CFs (75) (silage, cotton seed cake, brewer meal, maize meal, concentrates, compound feeds) in Limpopo, Mpumalanga, KwaZulu Natal, Gauteng provinces in South Africa. Feed samples were cultured for fungal isolation and DNA extraction done for PCR. In addition, extraction was done for mycotoxins using ELISA. The identification was done using Immunoaffinity column for HPLC and TLC.

Results: The results obtained showed that feed samples from both sources were contaminated with some of the major toxigenic fungal species; in the case of RSFs (% incidence): *Aspergillus flavus* (87%) *A. parasiticus* (43%) *A. niger* (69%) *A. ochraceus* (42%) *A. candidus* (23%) *F. verticillioides* (98%); *F. graminearum* (67%) and *P. verrucosum* (48.9%) and in CF samples: *A. flavus* (98%) *A. parasiticus* (51%) *A. ochraceus* (65%) *A. niger* (31%) *A. candidus* (21%) *F. verticillioides* (68%) *F. graminearum* (43%) and *P. verrucosum* (7%). Results of mycotoxin contamination showed the incidence of fumonisins (FBs) in RSF and CF samples of 90.6% and 93.3%, respectively with mean values of 1136.5ppb and 1045.5ppb; and aflatoxin (AF) contamination was 92.0% in RSF samples and 96.2% in CF samples with mean concentrations of 168.8ppb and 294.1ppb, respectively. The incidence rate of ochratoxin A (OTA) in RSF and CF samples were 85.4% and 83.7% with mean concentrations of 67.6ppb and 89.4ppb, respectively with zearalenone (ZEA) mean concentrations of 43.5ppb and 62.7ppb having an incidence of 50.6% and 55.3%, respectively. In addition, a co-occurrence of fungi and mycotoxins was found in both RSF and CF samples with 50.5% of RSF and 53% of CF samples being contaminated with all four analysed mycotoxins, whereas, 81.2 % and 79.5% of samples were contaminated with FBs, AFs and OTA mycotoxins simultaneously.

Conclusions: Although this study shows that both RSF and CF feed contained toxigenic fungal spp. and mycotoxins at similar frequencies and levels, all concentrations obtained for mycotoxins were above acceptable limits and thus of impact on animal health risk for human consumers. In addition, the co-occurrence of fungal strains in same samples and mycotoxins in animal feed raised the issue of additive and synergistic effects on animals.

Toxicology

P04-004-200

An unusual case of Paraquat poisoning in two calves

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Objectives: Paraquat, a bipyridyl herbicide, is widely sold for the control of broadleaved weeds. Toxicity in animals usually occurs after oral exposure. Paraquat is selectively toxic to lung where it accumulates in type I and II pneumocytes. The superoxide produced causes lipid peroxidation and necrosis. In animals that survive the acute phase, attempted repair results in progressive pulmonary fibrosis. Large doses may cause alveolar oedema, kidney and liver toxicity. There are numerous reports of paraquat toxicity in humans, dogs, cats and pigs but few in farmed animals. This report describes paraquat poisoning in two 10-week-old calves.

Materials and Methods: Two 10-week-old animals from a batch of 10 that were suffering from longstanding ill-thrift but presented acutely as recumbent and moribund were referred to Kilkenny Regional Veterinary Laboratory. The herdowner had raised concern about deliberate poisoning of the animals by a third party.

Full necropsy was performed. Sections of lung, liver, kidney and brain were processed by haematoxylin and eosin for microscopic examination. Lung, liver and faeces were cultured on xylose lysine deoxycholate (XLD) and ovine blood agar at 37°C. Liver and kidney were tested by atomic absorption spectrophotometry for copper and cobalt, lead and selenium respectively. Lung was tested for BHV-1, BRSV, PI3, BVD and BoCo viruses by real-time polymerase chain reaction (rt-PCR).

Liver and kidney samples from these calves, as well as samples of, water and feed to which these animals had access were tested by liquid chromatography & mass spectrometry (LCMS) at the State Laboratory, Backweston.

Results: Grossly the lungs were oedematous and congested. Grain was found in the ruminal contents, in one calf, approximately 5% of the grain was stained blue-green in colour. No other significant gross findings were present. Histopathological changes were most pronounced in the sections of liver and indicative of a severe acute to subacute, toxic hepatopathy in both animals (diffuse feathery degeneration, lipidosis, severe apoptosis in zones 1 and 2, mild biliary hyperplasia) and toxic nephropathy in one animal (diffuse lipidosis of renal tubular epithelium). Pulmonary changes were non-specific and primarily inflammatory in nature.

Paraquat was identified at apparently high concentrations in tissues from these two animals. Concentrations of the order of 12.9ug/g and 22ug/g in liver and 76ug/g and 166ug/g in kidney in the respective animals were determined. Chemical testing of water and feed, to which animals had access, were quantified at concentrations of the order of 2700ug/ml and 233ug/g respectively.

Findings from all other test results were insignificant and not considered a contributory factor in death of these animals.

All ten calves in the batch were reported by the referring veterinary practitioner to have died over the course of nine days, and none entered the food chain.

Conclusions: A diagnosis of paraquat poisoning was made based on demonstration of internal exposure, presence of a toxic hepatopathy and estimation of dose. A lethal dose of paraquat for cattle is considered to be 50mg/kg BW herbicide *per os*. In this case the dose rate based on consumption of contaminated water was estimated at between 288 and 315mg/kg BW.

Paraquat toxicity is biphasic, initially causing hepatic and renal insufficiency and pulmonary oedema followed by pulmonary fibrosis. The lack of pronounced lung pathology such as fibrosis normally associated with paraquat poisoning, may be due to the acute nature of death post exposure in this case.

Toxicology

P04-004-201

Monitoring Natural Feed Contamination Of Mycotoxins In Cattle: Metabolite Profiling Using Gas Chromatography-Mass Spectrometry

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Objectives: We previously reported a cattle herd that had an extensive contamination of feed with multiple mycotoxins such as zearalenone (ZEN) and sterigmatocystin (STC), and proposed to create a database for assessing potential risk factors for cattle health (Takagi et al., 2014 in WBC). Mass-spectrometry combined with gas chromatography (GC/MS) is a potentially useful tool for metabolomics, because of its high sensitivity, peak resolution and reproducibility. The objective of the present study was to evaluate the metabolic profiling of the cattle fed with or without ZEN/STC contaminated diets using GC/MS metabolomics approach.

Materials and Methods: Urinary samples were collected from individual animals (n=6 per herd) of fattening Japanese Black (JB) cattle herds. Herd 1: known to have persistently high urinary ZEN/STC concentrations due to the contaminated rice straws (ZEA; 7.5 mg/kg, STC; 0.24 mg/kg). Herd 2: a second fattening herd, which had a same feeding pattern as Herd 1, except for the rice straw (ZEA; LOD, STC; < 0.01 mg/kg). Herd 3: JB breeding cattle herd, with a different composition meeting the requirement of a breeding herd (ZEA; average 0.06 mg/kg, STC; not test). Additionally, urine samples were collected from Herd 1 two weeks after replacing the newly un-contaminated rice straw in feed (denoted Herd 1N: ZEA; LOD, STC; 0.04 mg/kg). Metabolites were extracted from the urine, derivatized and analyzed by GC/MS. Identified metabolites were subjected to ANOVA and principal component analysis (PCA). The variations in the amounts of the metabolites of each group were compared.

Results: Four groups were separated along PC1, confirming that the effects of ZEN/STC depend on exposure levels. The PCA results indicate that the metabolic profiles variation in each group differs. Typical variations were the amounts of glycine conjugates, which tended to be higher in Herds 1 and 1N than in Herds 2 and 3. This finding suggests an increased hepatic activity associated with detoxification. Similarly, the amounts of metabolites associated with gluconeogenesis and taurine metabolism tended to be higher in Herds 1 and 1N than that of Herds 2 and 3, which may suggest increased energy utilization in response to the stress condition in liver induced by the contaminants. The concentration

of hippurate that is clearly higher in Herd 3 than herds 1, 1N and 2 may be due to different dietary roughage content.

Conclusions: Our results indicate that the contamination of cattle feed with multiple mycotoxins such as ZEN and STC may result in an altered glycine metabolism and gluconeogenesis when compared with non-contaminated cattle. This first approach also suggests that monitoring endogenous metabolites in urine samples (metabolomics) is a useful tool to evaluate the effects of exposure of cattle to multiple mycotoxins. Further field studies should be carried out to create a database for the assessment of multiple mycotoxins as risk factors for cattle health and productivity.

Toxicology

P04-004-202

Hepatic lesions compatible with Pithomycototoxicosis (Facial Eczema) in cattle of São Miguel and Terceira Islands – Azores, Portugal

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Objectives: Pithomycototoxicosis is an intoxication caused by sporidesmin (SPD), a mycotoxin produced by fungus *Pithomyces chartarum*. Animals like sheep or cattle are more likely to develop hepatobiliary lesions when intoxicated. This disease was first located in New Zealand during the last half of 19th Century. In Azores, in the beginning of the millennium, SPD was identified as one of the causes of photosensitivity. The goal of this study was the establishment of the prevalence of calved cows with hepatic lesions compatible with Pithomycototoxicosis (HLCP), the age of animals, the degree of lesions and their impact on carcass evaluation.

Materials and Methods: Liver inspection was done during meat inspection on slaughterhouse line [abattoir from São Miguel Island (SMI) and Terceira Island (TI)] where liver was evaluated by observation and palpation. Livers with HLCP were categorized in four grades of severity [I (GI)-mild, II (GII)-moderate, III (GIII)-intense, IV (GIV)-severe]. Hepatic samples of each group were collected, fixed in 10% buffered formalin and submitted to routine histopathological analyses for microscopic confirmation of probable diagnosis. Collected data were used to determine the hepatic rejection rate (HRR), approbation rate (AR) and rejection rate (RR) of carcasses.

Results: In SMI were observed 1,331 livers. The average age (AA) of cattle was 80.34±29.847 months (m) and average weight (AW) was 261.34±49.491Kg. 23.37% of 1,331 livers had HLCP and this sample group showed AA of 86.49±29.817m and AW of 255.76±48.263Kg. 8.64% of the sample with HLCP exhibited GI lesions, 7.21% GII, 5.71% GIII and 1.80% GIV, with AA of 79.83±26.306m, 89.04±30.311m, 93.24±32.362m, 86.83±30.721m and AW of 262.89±49.160Kg, 257.93±50.147Kg, 248.80±45.734Kg, 234.92±36.377Kg, respectively. The AR of carcasses from total sample was 77.46% and the RR was 8.79%. The HRR of total sample was 97.75%, being the main causes of rejection telangiectasy (26.44%), fibrosis/sclerosis (F/S) (24.75%). In TI were observed 410 livers. The AA of cattle was 81.28±32.551m and AW was 259.09±42.418Kg. 29.51% of 410 livers had HLCP and this sample group showed AA of 93.16±29.741m and AW of 256.86±42.331Kg. 13.66% of the sample with HLCP exhibited GI lesions, 10.24% GII,

4.63% GIII and 0.98% GIV, with AA of 90.70±31.159m, 95.67±28.237m, 96.11±31.186m, 87.25±23.372m and AW of 260.54±40.477Kg, 261.91±46.386Kg, 241.47±37.828Kg, 225.25±14.338Kg, respectively. The AR of carcasses from total sample was 89.02% and the RR was 3.66%. The HRR of total sample was 69.02%, being the main causes of rejection the telangiectasy (24.03%), the F/S (23.32%). Meat Inspector (MI) can indicate only one hepatic rejection cause however livers can show more than one signal of pathology, what can only justify 65.1% (SMI) and 38.2% (TI) of the rejected livers with HLCP were, effectively, rejected because of F/S by MI.

Conclusions: The prevalence of cows with HLCP represents almost 1/4 to 1/3 of the sample. Worst lesions appear on older cows (SMI R=0.154; TI R=0.288), except in cows with GIV lesions. Maybe cows with worst HLCP have reduced life time. With increasing HLCP grade carcass weight decreases (SMI R=-0.089). Lighter cows are more prone to SPD action presenting worst lesions or cows intoxicated with SPD have less metabolic ability, resulting in lighter carcasses. Pithomycotoxicosis may be underestimate since there are livers with HLCP not rejected by F/S, this happens mainly in livers with lower grades of HLCP and signals of diverse diseases.

Toxicology

P04-004-203

CASE REPORT: Monensin Intoxication in Bubalus bubalis Calves

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Objectives:

1. To define the cause of massive sudden death of calves in a Bubalus bubalis exploitation in the Northern Pacific region of Costa Rica.
2. To establish a therapeutic management to recover the affected animals.
3. To recommend a proper management to control future loses.

Materials and Methods: The assessment included a complete evaluation of the history and records, physical examination, field inspection, toxicological evaluation of pastures to assess the content of Cyanide and Nitrates, analysis of the levels of methahemoglobin, Complete Blood Counts, Total Protein levels measurement, Creatine phosphokinase levels measurement, electrocardiographic examination, ecocardiographic examination, necropsies and histopathology. Animals with and without clinical signs compatible with the traditional presentation of the deaths were evaluated.

Results: It was found that the animals affected were only the recently weaned. All animals were eating in similar pastures. The weaning calves were administered a cattle calve concentrate with 60 mg/kg of monensin. Other animals were given a lactating cow concentrate with 30 mg/kg of monensin. The weaning group had a bad body condition score, hirsutism, weakness and anemia. The affected animals that had not died were tachypneic, open mouth breathing and with foam in the mouth. High levels of cyanide and nitrates were found in the pastures. Although the clinical signs were compatible with these intoxications, the specific treatment did not show a consistent response. The animals kept

dying even when they were moved to other pastures with lower levels of cyanide and nitrates. The electrocardiographic findings showed an increase in the height and length of the P wave of the affected animals. The ecocardiographic findings showed a decrease in the width of the cardiac walls of the affected animals. Three necropsies were performed. The findings showed paleness of the miocardic muscle and skeletal muscles. A necrotizing lymphohystiocitic miostis from low to moderate was found in the heart and skeletal muscle. A semitendinosus and semimembranosus biopsy was taken in an affected lying animal. The hystopathology revealed a severe necrotizing lymphohystiocitic miostis. The levels of CPK in this animal were elevated. The substitution of the concentrate with other with no monensin stopped completely the mortality in the weaning group of the farm.

Conclusions: It was concluded that the monensin in an estimated range from 0.6 – 0.8 mg/kg could be toxic in Bubalus bubalis. The monensin in Bubalus bubalis behaves as a myotoxic agent. Nevertheless there were evidence of a presence of cyanide and nitrate in the pastures, it was not the primary agent affecting the animals.

Tuberculosis

P01-001-166

1. Advances in monitoring of tuberculin testing in the Irish bTb eradication programme 2008 - 2014

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Objectives: This study sets to evaluate the impact of enhanced quality controls on key performance indicators of Single Intradermal Comparative Cervical Tuberculin Test (SICCT) performance during the period 2008 to 2014 using analysis of data captured in specialist reports on testing details of individual PVPs. These data facilitate monitor of individual and collective delivery of the SICCT by Private Veterinary Practitioners (PVPs).

Materials and Methods: A specialist report using the Department of Agriculture Animal Health Computer System (AHCS) was introduced in 2008 to monitor the performance of each PVP in delivery of the national bovine tuberculosis (bTb) eradication programme. The report captures all data relevant to each PVP and concentrates on Key Performance Indicators (KPI) that affect the quality of both administrative and field performance. The KPIs enable comparison of PVP performance over time and between peers. The key measures of performance are critical control points which were chosen because they are objective and readily measurable. Since the introduction of the reports in 2008 there have been considerable changes in on individual tester performance both in relation to programme delivery and disease detection performance. The data recorded all tests conducted by PVPs on cattle in Ireland from January 2008 to December 2014 including dates, circa 116,000 herds and test readings for circa 8.5 million animal tests per annum. Statistical analysis was performed on numbers of herds tested, animals tested, clear animals reactors identified as reactors within six months at a subsequent test, abattoir cases detected in clear animals within six months of test, amendments to details of tests previously certified, late test report submissions and non-compliance with advance itinerary requirements. All tests were included regardless of geographic location, herd size or local area prevalence of bTb.

Results: These data indicate that the total number of PVP engaged in testing has not changed considerably from 947 in 2008 to 970 in 2014. The mean number of animal tests has decreased from 8,696 in 2008 to 8,224 in 2014 mainly due to a decrease in disease incidence and reduction in consequential testing of infected and contiguous herds. The mean number clear animals tested by each PVP which were identified as reactors within six months at a subsequent test declined from 2.09 in 2008 to 1.22 in 2014. The mean number of confirmed abattoir cases detected within six months in animals tested clear fell from 2.11 in 2008 to 1.03 in 2014. Submissions of subsequent amendments to test details for tests previously certified reduced from a mean of 3.1 in 2008 to 1.1 in 2014. Late test report submissions reduced from a mean of 16.3 in 2008 to 3.2 in 2014. Non compliances with advanced itinerary requirements reduced from a mean of 25.5 in 2008 to 6.7 in 2014

Conclusions: The decline in back trace detection test positive and disclosure of TB lesions at slaughter of recently tested clear animals indicate improvement in disease detection. The frequency of amendments to certification (AHCS 1 submissions), conformity with advanced itinerary submission and punctuality of test report submissions are independent of disease prevalence and therefore reflect change in behaviour by PVPs. The data indicate an improvement in the quality of testing by PVPs since the introduction of the specialist reports in 2008 and is likely to be a factor in the concurrent reduction in disease prevalence.

Tuberculosis

P01-001-168

Improved robustness of bovine Tb testing using a second generation Interferon Gamma Release Assay based on peptide antigens and In-tube technology

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Objectives: For human Tb-diagnostics, Interferon-gamma release assays (IGRA) based on modern peptide antigens like QIAGEN's QuantiFERON-TB Gold In-Tube (QFT-IT) has become the test of choice in screening for TB infections. But in veterinary diagnostics, in particular for bovine TB testing, IGRAs are not yet widely accepted. The study presents data on improved robustness and convenience of bovine Tb testing using a modern peptide-based In-tube IGRA technology.

Materials and Methods: While IGRAs become more and more the Golden standard for diagnosis of Tb in humans, this method is not yet well accepted in diagnostics of bovine Tb yet. There are concerns about the accuracy of test results obtained with available first-generation PPD-based IGRAs. A specificity range of 87.7 to 99.2% was reported, which causes problems in low prevalence areas.

New peptide antigens were successfully validated in bovine Tb research studies but were not yet available in easy to use diagnostic kit format. These peptide antigens show higher specificity without marked loss of sensitivity, no cross-reactivity in paratuberculosis-infected herds and potential to differentiate vaccination using BCG vaccines from bovine TB infection.

QFT-IT is an advanced blood test and represents a new generation of peptide-based IGRAs that are more accurate, faster, and cost-effective than older tests and other methods. The assay measures the cell-mediated immune response of TB-infected individuals.

QFT-IT uses special sample collection tubes with peptide antigens that are coated directly on the tube walls. Those vacuum tubes allow the collection of 1ml of blood sample during the bleeding process. Incubation of samples in these tubes eliminates the need for expensive shipping samples at 37°C in a narrow time window. After a stimulation step for 24h at 37°C plasma from the TB antigen tube and Nil tube is tested with the cattletype® IFN-gamma ELISA.

Plasma samples can be tested manually or automated using the DS2® ELISA Processing System or the DSX® ELISA Processing System (available from QIAGEN).

Results: In order to improve the logistic pathways for samples, a robustness study was performed for the QFT In-tube method. 1 ml aliquots of blood samples from skin test positive animals were transferred to 3 QFT TB tubes and 1 Nil tube. One Tube was held at 4°C for 24 h and another tube was held at 20°C for 24 h prior to the 37°C stimulation incubation. Results obtained with the previously stored tubes were compared to the results obtained with the standard procedure.

The sample storage at 4°C showed no ponderable impact on the test results (average S/P values +2.4%). When stored at 20°C for 24 h S/P signals decreased on average 24.6%, but no false negative results were observed compared to the standard method.

Conclusions: A new generation of IGRA using modern antigens in a user-friendly In-tube format as described here has the potential to overcome the obstacles of lack of robustness and specificity and for a new quality of Tb testing in veterinary diagnostics.

Tuberculosis

P01-001-169

The bovine tuberculosis risk conferred by the purchasing herd post movement.

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Objectives: Bovine tuberculosis (bTB) is subject to a compulsory eradication programme in Ireland. The programme operates under national legislation and fulfils the requirements of the relevant European Union Trade Directives. Movement restrictions form a key component of the programme. In excess of 2.8 million farm to farm bovine movements are recorded annually. The aim of this study was to estimate the risk of a positive bovine tuberculosis (bTB) skin test conferred by the purchasing herd post movement.

Materials and Methods: Data were gathered from the Animal Information and Movement System (AIMS) database corresponding to cattle movements ($n = 402,365$) into herds that subsequently had a bTB breakdown between 2011 and 2013. A backwards stepwise multivariable logistic regression model was developed to evaluate the independent factors; year, animal age, sex, movement to test positive interval, and purchasing herd factors including herd size, location, management, and test-type, accounting for herd structure, on the risk of becoming positive to the bTB skin test.

Results: Only 0.44 % of animals moved into herds that subsequently breakdown with bTB are skin test positive within two months. While, 0.95 % of animals that moved herds within the past year subsequently test positive to the bTB skin test. The odds ratio for a positive bTB skin test increased ($P < 0.001$) in animals tested more than two months after a move. Animals were 1.32 times (confidence interval; CI 1.13 to 1.54) more likely ($P < 0.001$) to be bTB skin positive if tested more than two months post move compared with those tested within two months of a move (referent level). The risk of disclosing a bTB positive animal was greater ($P < 0.001$) the smaller the purchasing herd. The location of the purchasing herd altered the bTB reactor risk, with purchased animals in herds located in areas with high infection pressure two times (CI 1.64 to 2.42) more likely ($P = 0.004$) to be positive to the bTB skin test compared with animals in herds located in low risk areas. The management choice to purchase through a public market lowered ($P = 0.004$) risk compared with purchase direct from farm.

Conclusions: Although it is widely accepted that the seller herd influences the bTB risk, these data provide evidence that purchasing herd size, location and method of purchase alters bTB risk. In conclusion, the purchasing herd confers a substantial risk of an animal becoming bTB positive following movement.

Tuberculosis

P01-001-170

Monitoring the effectiveness of Irish factories in the surveillance of slaughtered cattle for tuberculosis lesions: An update for years 2008-2010

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Objectives: In Ireland, every animal is examined at slaughter for its fitness for human consumption. The detection of tuberculous lesions at slaughter has proved to be an essential component of the bovine tuberculosis (TB) surveillance system for the cattle population. The aim of this study has been to monitor and determine the relative effectiveness of factories in submitting and subsequently in having suspect lesions confirmed as bovine tuberculosis (TB) lesions from cattle from attested herds (i.e. herds classified free of bovine TB). Thus, this study provides an update for the period 2008-2010 that serves as comparison to previous years (2005-2007).

Materials and Methods: Data were obtained from the Department of Agriculture Food and the Marine (DAFM), Ireland. Factory data regarding number of animals slaughtered, numbers of bovine TB lesions submission and laboratory confirmation results were evaluated for all export-licensed factories in Ireland. Additionally, animal movement and TB tuberculin test results were obtained. A Multivariable logistic regression model was developed to calculate the risk of lesion submission (and separately confirmation risk) for each factory, using animal as the unit of analysis. The model included the following potential confounding factors: the sex, age and origin of each animal, the slaughter season, and, for each animal's herd of origin, the length of the TB clear period for the animal source herd and the District Electoral Division (DED) bovine TB risk. The adjusted and crude lesion submission (and separately confirmation) risks were compared to determine the effect of this adjustment on the estimates of risk on the ranking of the factories.

Results: Our results show that for the period 2005-2007, the observed (crude) factory submission risk ranged from 0 to 52 per 10,000 animals slaughtered. There was a five-fold range in lesion submission risk among Irish factories. The average crude submission risk (25 per 10,000) during 2005-07 was higher than found in previous years (21.8 per 10,000; 22 per 10,000). For the period 2005-2007, the variation in crude submission risk among factories 5-fold was lower than reported previously, which was lower than the 7-fold difference and a 9-fold difference found in previous studies before 2005. For the period 2005-2007 the factory confirmation risk ranged from 30.2% to 90.1%, with an average of 68.5%, and this average was slightly higher than the 64.4% reported for years before 2005. The data for the period 2008-2010 will be analyzed and presented in July 2016.

Conclusions: Our studies over the years have shown substantial variation in the effectiveness of lesion submission and subsequent confirmation as bovine TB among Irish factories. Our updated analysis for the years 2008-2010 will provide important information to managers to assess progress towards reducing the variability in submission and confirmation risk for bovine TB. Thus, this study is important as part of quality control in the national bovine TB programme.

Tuberculosis

P01-001-171

Assessment of an advanced interferon- γ assay under field conditions of Piedmont region (Italy)

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Objectives: BOVIGAM® is a ELISA kit certified by the OIE to control bovine tuberculosis in cattle, showing good sensitivity and specificity. Piedmont region is obtaining officially bTB free status after 14 years of a strategic application of g-interferon assay (g-IFN) as ancillary test of Skin Intradermal Test (SIT), that shows under field conditions high specificity but lower sensitivity respect to γ -IFN. In order to improve diagnostic performance, optimizing local bTB eradication program, an advanced g-IFN (BOVIGAM® 2G) was evaluated under field trial conditions.

Materials and Methods:

Herds were selected:

- bTB free herds with SIT negative results in order to evaluate specificity;
- bTB infected herds with SIT and/or interferon- γ assay positive results and *M. bovis* culture and/or PCR positive in order to evaluate sensitivity.

All collected animals resulted:

- not vaccinated with BCG vaccine;
- older than 6 month;
- without respiratory diseases at the moment of sampling.

The specificity of BOVIGAM® 2G was calculated collecting and analyzing 176 blood samples from 8 different bTB free herds. For each samples was performed simultaneously BOVIGAM® and BOVIGAM® 2G ELISA. In order to assess sensitivity, 109 animals have been collected from 15 different bTB infected herds. The whole blood stimulation was performed with Avian and Bovine Italian PPDs (Purified Protein Derivative), Avian and Bovine Lelystad PPDs, Peptide Cocktail E-C (Mix A) with high specificity and ideal for monitoring purposes, Peptide Cocktail H-P (Mix B) with high specificity and sensitivity useful during eradication programs and Pokeweed Mitogen. BOVIGAM® 2G ELISA was carried out in two different ways using either manufacturers' interpretative criteria than those described by Dondo et al. . Sensitivity, specificity and agreement for each test and criteria were evaluated using WinEpi© tool (Ignacio de Blas. Facultad de Veterinaria, Universidad de Zaragoza, 2006).

Results: 38 samples showing high Optical Density (O.D.) values for the nil antigen, reduced to 25 using an highest cut-off value, were removed from the study, due to an aspecific interferon γ production. For all samples, it needed also to reduce the conjugate incubation time in order to avoid overflow values and allow the correct OD values interpretation. This trial under field condition assessed BOVIGAM® 2G test has a sensitivity slightly more low than that obtained by BOVIGAM®, although not statistically significant. Otherwise, the specificity is good with values higher than 96% for all of antigens tested, except for MIX B (93.8%; IC: 90.1-97.3%). It was observed a good agreement in all of methods tested, obtaining KAPPA coefficient values ranging from 0.462 to 0.838; however the highest levels of agreement were between SIT and BOVIGAM® 2G.

Conclusions: bTB diagnosis is complex and requires in vita diagnostic tools flexible and form fitting to specific local situation. Our results indicate BOVIGAM® 2G improves specificity, that is useful during monitoring programs in bTB low prevalence region, such as Piedmont Region, where the study was conducted. More studies should be carried out in our region to better assess the use of both peptide cocktails (MIX A and MIX B) and define their strategic application in our bTB situation to optimize control actions.

Comments: The authors acknowledge Thermo Fischer Scientific for technical and scientific support including the supply of materials used in this trial.

Tuberculosis

P01-001-172

Validation of stimulants and interferon gamma ELISA for bovine tuberculosis detection

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Objectives: IGRA (Interferon Gamma Release Assay) is widely used to detect the cellular response to pathogens such as *Mycobacterium bovis* by measuring the difference between activated and non-activated whole blood interferon gamma (IFN γ) signals. IDvet offers a complete solution for the detection of bovine tuberculosis. It includes stimulation antigens and an ELISA kit for detection. The aim of this study is to assess the performance of this solution (sensitivity, specificity, repeatability, reproducibility, and stability).

Materials and Methods: PPDa and PPDb tuberculins (IDvet) were used as stimulation antigens. IFN γ level was then determined using the (ID Screen®) Ruminant Interferon Gamma ELISA (IDvet). All ELISA results were standardized and expressed as sample/positive control ratios (S/P). Different freeze-dried IFN γ reference materials were created from natural IFN γ from different species (bovine, ovine, caprine or buffalo). The stability, repeatability, reproducibility and robustness of the ELISA itself was studied. Specificity for bovine tuberculosis diagnosis was evaluated on cattle from different breeds and countries (n=1077). Sensitivity was evaluated in comparison with confirmatory techniques (PCR, single and comparative intradermal tuberculin test).

Results: The ELISA reagents showed high stability. Repeatability was excellent, with CV% values measured to be between 3 and 8%. Reproducibility was evaluated through the analysis of 60 dilutions of the freeze-dried bovine plasma standard by 4 French Departmental Laboratories in independent runs. S/P% values were between 55 and 68%, with a standard deviation (SD) of 3 and a coefficient of variation (CV %) of 5%: analytical sensitivity was found to be constant and equivalent between runs. Measured specificity was 99.0% (IC95 98.4 – 99.6%) and sensitivity was 88.3% (IC95 81.1 – 95.5%). Specificity was also high for Paratuberculosis-infected herds, even with high seroprevalence (Belgium: Sp= 98% and France, Midi-Pyrénées: Sp = 100%). Sensitivity with respect to confirmatory techniques was 88.3% (IC95 81.1 – 95.5%).

Conclusions: The use of the IDvet PPDs with the IDvet IFN γ ELISA gave a measured test sensitivity comparable to published values. The stability, repeatability and reproducibility of this ELISA was high. Thanks to the use of tuberculins with appropriate potencies, as well as well-defined interpretation criteria, test specificity was high. The performance of the IDvet PPDs and IFN γ ELISA meets bovine TB eradication program requirements.

Tuberculosis

P01-001-173

A study of Irish Farmers, whose herds were depopulated as a result of Bovine Tuberculosis, in the years 2008-2010

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Objectives: This study documents Irish farmers' experiences of herd depopulations due to Bovine Tuberculosis. Depopulation is used as a programme measure when the infection level in a herd has surpassed 30%. Between 2008 & 2010, 87 farms had a full or partial depopulation of their herd. These herds accounted for 0.078% of herds nationally restricted in the three year period, but for 7% of all animals slaughtered as a result of bTB in the same period.

Materials and Methods: These farmers were surveyed, by means of postal questionnaire, telephone and face to face interview. The questionnaire was designed so as to extrapolate from results whether policy is fit for purpose. The response to the postal questionnaire was poor and telephone interviewing was far more effective in collecting information. Two cases of human infection were described in the survey and reminds why eradication is a national target.

This study conducted exploratory and descriptive research on the experiences of a specific group of Irish farmers to gain an understanding of the effects of herd depopulation due to bTB. The researcher used the data set of Irish Cattle Farmers who lost part or their entire herd in the years 2008, 2009 & 2010 and had two primary goals:

- Document their experience of depopulation
- Extrapolate from the documentation whether overall policy was fit for purpose for the eradication of bTB

Results: The results describe farmers' experience of depopulation and show that it is accepted by farmers as a necessary instrument of the national eradication campaign, especially once infection levels within the herd reach a critical level. Most farmers restocked with bovines and found the purchased herd to be healthier than the one it replaced. There was no one single reason for the breakdown, but some important themes emerged in terms of what was considered the cause and how to prevent a reoccurrence. Interpretation of their experience of the depopulation and their interactions with actors associated with service delivery makes a compelling case that the overall policy is working, albeit with variation and some areas of concern. The main themes of contention were compensation, stress in the home and workplace (sometimes associated with illness) and the feeling of being out of control and having a limited input into decision making. Some distinctions emerge in terms of family farms and farms that are run as businesses. The survey results are used to suggest pathways for re-engaging with the farming community with a view to expanding their role in disease eradication and to encourage proactive participation at a farm and industry level. A basic conceptual model was constructed to compare successful eradication campaigns with non-successful programmes.

Conclusions: The forces that influenced depopulation events were major infective outbreaks of bTB occasionally accompanied with non-specific infection. The causes of the outbreaks were split evenly between wildlife and bovine sources. The contentious areas of current bTB eradication policy are compensation, effective wildlife control, bought in infection, and poor tuberculin testing. The working hypotheses that are warranted based on farmer experiences to guide further research, include insurance, farmer removal of wildlife, accelerated capital allowances for disease prevention, prosecutions of negligence and stakeholder partnership.

Udder Health & Milk Quality

P01-001-174

In vitro Growth Inhibition of mastitis-causing bacteria by *Lactobacillus Plantarum* ATCC 8014

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Objectives: In recent years, global demand for organic dairy products has increased rapidly. There has been a huge growth in number of dairy farms being converted to organic management systems. Because of restricted use of antibiotics in an organic dairy farm, researchers' attention has been drawn toward probiotics and their by-products as natural remedies potentially replacing antibiotics. Since mastitis is a serious problem in dairy industry, the aim was to evaluate antibacterial potency of the cell-free lactobacillus supernatant against mastitis-causing pathogens.

Materials and Methods: Six mastitis-causing pathogens (*Staphylococcus aureus*, *S. non aureus*, *E. coli*, *Klebsiella*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae*) were isolated from bulk tank milk. *Lactobacillus plantarum* ATCC 8014 was cultured in De Mann Rogosa sharpe (MRS) broth for 24h at 37 °C in a candle jar. Cell-free supernatant (CFS) was obtained by centrifuging the culture medium at 3000 rpm for 15 minutes and was sterilized by a 0.45µm filter. Growth inhibition was determined in micro plates. Wells were filled with 80 µl Tryptic Soy Broth (TSB, as a culture medium), 100 µl CFS and 20 µl of each of pathogenic bacteria with a concentration of 10⁸ CFU/ml. Three and 18 wells, respectively were allocated to negative (100 µl TSB +100 µl CFS) and positive (PC, 180 µl TSB + 20 µl of each pathogen) controls. Sealed plate incubated at 37 °C for 24 h then OD₅₄₀ was measured by a microplate reader. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined by lyophilized CFS and microdilution method. Final volume of each well was 75 µl. Wells without CFS and with pathogen bacteria used as positive controls, while negative control wells contained only culture medium (TSB). Microplates were incubated at 37 °C for 24 h. A wire loop of wells without visible growth cultured on the nutrient agar plates for determination of MBC. Each run were repeated in triplicate and average of runs were analyzed by SAS.

Results: Pathogen inhibitory potency of CFS of *Lactobacillus Plantarum* ATCC 8014 was confirmed (P <0.001). There was no visible growth in treatments receiving CFS. The OD₅₄₀ of PC and CFS-containing wells were 1.062 and 0.027, respectively (for *S. aureus*), 0.886 and 0.039 (for *E. coli*), 0.689 and 0.068 (for *S. non aureus*), 0.769 and 0.06 (for *Klebsiella*), 0.518 and 0.028 for (*S. agalactiae*) and 0.522 and 0.06 (for *S. dysgalactiae*). MICs and MBCs were similar for all pathogens (8.45 mg/ml) except for *S. non aureus*. MIC and MBC for *S. non aureus* were 4.22 and 8.45 mg/ml, respectively.

Conclusions: Results obtained in this study suggest that *Lactobacillus plantarum* metabolites can inhibit growth of six common pathogens prevalent in dairy cow mastitis. However, chemical characteristics of these metabolites were not known in this experiment and should further be analyzed. Also, a complete understanding of the mode of action of these metabolites might have practical implication in natural cure of clinical mastitis cases.

Keywords: Inhibitory activity, *Lactobacillus*, Mastitis

Udder Health & Milk Quality

P01-001-175

Quantifying Milking Frequency Effects On Quarter Milk Production Rate

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Objectives: The objective of this observational study was to quantify the association between milking frequencies and quarter milk production rate (kg/hr) for primiparous and multiparous cows on a commercial dairy farm using a robotic automatic milking system technology.

Materials and Methods: Eight months of milk harvesting data was sourced from a 1400 cow commercial dairy farm using multiple, single stall robotic milking units. Individual quarter milk production rates were calculated for primiparous and multiparous cows during the days in milk windows: DIM 50-80, 100-130, 150-180. Milk production rate was calculated for all front and rear quarters by taking quarter milk yield and dividing this by the inter-milking interval (rounded to the nearest hour) for that specific cow and milking event. Hence, this calculation was performed for each individual milking. More than 250 individual milking observations had to be collated for any specific inter-milking interval for the data to be included and the analysis went up to a maximum interval of 14 hrs. Pre-milking preparation (teat cleaning and pre-stimulation) and detacher settings did not alter between milkings. Cows were assessed only against a time based threshold for gaining milking permission when they presented to a robotic milking unit. The minimum time since the last milking for any animal in the herd to be given milking permission was 4 hours. Descriptive statistics on milking frequency were also collated. Only analysis on left front and rear quarters is presented as results on left front and rear quarters compared with right front and rear were closely aligned.

Results: Rear quarters in this study population produced between 27.4 and 31.1% of total milk yield at an individual milking with front quarters producing between 19.0 and 23.2%. Hence, the milk production rate at the quarter level is, typically, higher for the rear compared to the front quarters. In this analysis, a significant trend of reducing milk production rate was evident for both primiparous and multiparous cows in all three DIM windows, for all quarters, from 4 hrs to 14 hrs inter-milking interval. Primiparous cows had a lower milk production rate when compared to multiparous cows by approximately 19%. Between inter-milking interval 4 and 12 hrs, multiparous cows had a maximum milk production rate (kg/hr) of 0.57 at 4 hrs (left rear, DIM 50-80) and a minimum rate of 0.30 at 12 hrs (right front, DIM 150-180). Primiparous cows had a maximum milk production rate of 0.43 at 4 hrs (left rear, DIM 50-80) and a minimum rate of 0.30 at 12 hrs (left front, DIM 150-180). The mean inter-milking interval for the 672 multiparous cows in this analysis was 9.2 hrs with a standard deviation of 1.9 hrs. For the 299 primiparous cows the mean was 8.3 hrs with a SD of 2.1 hrs.

Conclusions: This study has determined that gains of approximately 1.5% in milk production rate at the quarter level are associated with each decrease of an hour in the inter-milking intervals between 4 and 14 hrs. This trend is demonstrable for both primiparous and multiparous cows and is reasonably consistent across all three DIM windows analysed for all front and rear quarters.

Udder Health & Milk Quality

P01-001-176

Mammary epithelial cells, rather than professional immune cells dictate the pathogen-species specific immune reaction of the udder during mastitis

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Objectives: The outcome of an udder infection (mastitis) largely depends on the species of the invading pathogen. Gram-negative pathogens, such as *Escherichia coli* often elicit acute clinical mastitis while Gram-positive pathogens, such as *Staphylococcus aureus* or *Streptococcus uberis* tend to cause milder subclinical inflammations. It is unclear which type of the immune competent cells residing in the udder governs the pathogen species-specific physiology of mastitis and which established cell lines might provide suitable models.

Materials and Methods: We profiled the pathogen species-specific immune response of different cell types derived from udder and blood. Primary cultures of bovine mammary epithelial cells (pbMEC), mammary derived fibroblasts (pbMFC), and bovine monocyte-derived macrophages (boMdM) were challenged with heat-killed *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus uberis* mastitis pathogens and their immune response was scaled against the response of established models for MEC (bovine MAC-T) and macrophages (murine RAW 264.7).

Results: Only *Escherichia coli* provoked a full scale immune reaction in pbMEC, fibroblasts and MAC-T cells, as indicated by induced cytokine and chemokine expression and NF- κ B activation. Weak reactions were induced by *Staphylococcus aureus* and none by *Streptococcus uberis* challenges. In contrast, both models for macrophages (boMdM and RAW 264.7) reacted strongly against all the three pathogens accompanied by strong activation of NF- κ B factors. Hence, the established cell models MAC-T and RAW 264.7 properly reflected key aspects of the pathogen species-specific immune response of the respective parental cell type.

Conclusions: Our study shows that the models for mammary epithelial cells and fibroblasts, but not macrophages respond with distinctly graded immune reactions against each of the three pathogens. *Escherichia coli* but neither of the Gram-positive bacteria elicits in them synthesis of a strong and transient cytokine storm. This distinction is partly caused by the failure of MEC to activate TLR-mediated signaling upon challenges with *Staphylococcus aureus* or *Streptococcus uberis*. Hence, the pathogen species-specific norm of the immune response of MEC rather than that of macrophages appears to dictate the immune response of the udder early on after infection.

Udder Health & Milk Quality

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Risk Factors For Peri-Parturient Farmer Diagnosed Mastitis In Canterbury Dairy Herds: Findings From A Retrospective Cohort Study.

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Objectives: To investigate cow level risk factors for peri-parturient cases of farmer diagnosed, clinical mastitis (CM) in spring calving pastoral based New Zealand dairy herds using a retrospective cohort study.

Materials and Methods: A retrospective longitudinal cohort study of 18,162 cows on 30 spring calving South Island New Zealand dairy farms was used to identify factors associated with the development of the first case of clinical mastitis in multiparous cows from 30 days before to 90 days after calving. All cows were dried off over the last two weeks of May 2012 using cephalonium dry cow therapy (Cepravin Dry Cow, MSD Animal Health, Upper Hutt, New Zealand).

Clinical mastitis was diagnosed by farm staff using changes in the udder, milk, or generalised illness.

Risk factors studied included age, breed, length of dry period, farm of origin, herd size, yield and cell count status 30-60 days before the end of the previous lactation, rainfall at calving and number of animals calving on the same day.

The effect of cell count status at the herd test before drying off, age, length of dry period and breed varied with time from enrolment. Plots of the cumulative estimated regression coefficients from an Aalen linear survival-time model were used to identify the form of the time varying effects.

A modified Cox Proportional hazards model with time varying effects for breed, age, length of dry period and cell count status 30-60 days before the end of the previous lactation was constructed by splitting the dataset at calving and 20 days after calving. For each of the time varying covariates, three covariates were created to reflect the varying effects of the predictor: early (30 days before calving to calving), mid (calving to 19 days post calving) and late (20 to 90 or more days after calving).

Results: Rainfall at calving, breed, age, length of dry period, yield and cell count status 30-60 days before the end of lactation were identified as significantly associated with an increased hazard of clinical mastitis around calving. Number of animals calving per day and herd size were not associated with an increased risk of clinical mastitis and there was a significant difference in the hazard of mastitis across farms.

The hazard of mastitis was 1.34 times higher for cows giving >1.5kgMS at the last herd test of the preceding lactation (95%CI=1.21-1.52) and 1.13 times higher if rainfall \geq 10mm on the day of calving (95%CI=1.01-1.30). Cross bred cows were predicted to have a lower hazard for CM with the hazard decreasing as the proportion of Jersey genetics increased. A cell count exceeding 150,000 cells/ml at the last herd test of the preceding lactation increased the hazard of CM by 1.60 (95%CI=1.39-1.84) in the first 20 days after calving and 1.95 (95%CI=1.67-2.27) 20-90 days after calving. The model predicted that compared to young (age=3 yrs) cows, cows aged 4-8 years had an elevated hazard of CM after 20 days in lactation (2.18, 95%CI=1.71-2.76), while cows older than 8 had an elevated hazard in all time periods at 2.98, (95%CI=1.73-5.14) before calving, 1.74 (95%CI=1.39-2.18) in the first 20 days of lactation rising to 4.09 (3.06-5.46) after 20 days.

A dry period of 112 days or more increased the hazard of mastitis in the first 20 days after calving 1.46 times (95%CI=1.24-1.73) but reduced the hazard 0.73 times for days 21-90 (95%CI=0.57-0.94).

Conclusions: This study confirms that the period around calving is the peak time for clinical mastitis diagnosis. In this study, 11% of multiparous cows were diagnosed with clinical mastitis from 30 days before to 90 days after calving and 38% of these cases were diagnosed within one week of calving. Only 3.4% of all cases were diagnosed during the dry period, affecting 0.4% of all cows. Although cow level studies can be useful in identifying variables that operate at this level

much of the variation in incidence of clinical mastitis stems from higher level variables operating at the herd level.

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Molecular characterization of coagulase negative staphylococci from heifer intramammary infections and potential body site reservoirs

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Objectives: Coagulase negative staphylococcal (CNS) species are a common cause of subclinical mastitis in dairy heifers (De Vliegher et al., 2012). Currently, the relationship of body site colonization with these organisms and intramammary infection (IMI) in heifers is not well understood. The objective of this project was to determine if specific body sites were colonized with the same CNS species and strain type that were causing CNS IMIs in heifers.

Materials and Methods: Primiparous heifers (n = 100) at the University of Missouri Foremost Dairy were enrolled in the study. Pre-calving samples were collected at approximately 14d prior to expected calving date and included mammary quarter secretions and body site swabbing samples of teats, muzzle, perineum, and inguinal region. Swabbing samples were collected using gas sterilized electrostatic dusters (Swiffer®). At calving, mammary quarter foremilk samples were collected twice, at approximately 3-5 and 7-9 days postpartum, for bacterial culture and somatic cell counting. All body site swabbing samples were chilled on ice for transport and processed the same day. Swabbing samples were mixed with 10 ml of sterile saline, agitated, and plated on Mannitol Salt Agar (MSA). At 24h, plates were read and up to 10 staphylococcal colonies, including at least one of each morphologically distinct colony type, were sub-cultured onto Columbia Blood Agar (CBA). For all pre-partum secretions and milk samples, a 0.01 ml aliquot was plated onto CBA and primary identification was determined according to the National Mastitis Council guidelines (Hogan, 1999). Samples were considered to be culture positive if ≥ 1 colony were observed (≥ 100cfu/ml). All staphylococcal isolates were banked in phosphate buffered glycerol at -80°C, and later speciated using DNA sequencing of the *rpoB* housekeeping gene (Drancourt and Raoult, 2002) and matrix-assisted laser desorption ionisation-time of flight (MALDI-ToF) mass spectrometry. Strain-typing was done using pulsed-field gel electrophoresis.

Results: The most common organisms identified at all three sampling points were CNS species. The most common CNS species identified in both pre-partum and post-partum samples was *Staphylococcus chromogenes*. Other common species identified were *S. agnetis* and *S. simulans*. A total of 2357 staphylococcal isolates were recovered from heifer body site samples. To date, 29% (682/2357) of these isolates have been speciated. After removing species represented more than once per body site sample for each heifer, a total of 241 of the 682 isolates have been characterized. The most commonly identified species from these body site samples were *S. chromogenes*, *S. haemolyticus*, and *S. devriesii*.

To date, PFGE strain-typing has been completed on all *S. chromogenes* isolates from five heifers. Multiple strains of *S. chromogenes* have been identified on all heifers characterized thus far. *Staphylococcus*

chromogenes strains isolated from the mammary gland were found to be 100% similar to a teat skin isolate on 3/5 heifers, to an inguinal isolate on 1/5 heifers, and to a teat skin and inguinal isolate on 1/5 heifers.

Conclusions: CNS is a common cause of subclinical mastitis in dairy heifers. In this study, *S. chromogenes* was the most common cause of CNS IMI, and was also commonly found in the inguinal region of pre-partum heifers. To date, preliminary strain-typing data has shown that *S. chromogenes* isolated from the mammary gland can be related to inguinal and teat skin strains. These body sites could be important sources of *S. chromogenes* for heifer IMI. Final results of this project will further the understanding of the epidemiology of CNS infections in dairy heifers and allow for the development and study of methods to control and prevent IMI.

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Udder Health & Milk Quality

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A new and simple method to deal with teat injuries

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Objectives: Teat injuries are common in dairy cattle, around 2-3% are affected^{1,2}. They can be divided into two categories (external or internal injuries). They often result in premature culling of affected cows.

Surgery and treatment of these injuries has been described and discussed intensively^{3,4}. However the question whether complete rest (no milking) or temporary resting of the injured teat by temporary cessation of milking⁵ or continuous milking is not yet clearly answered and may vary from case to case.

This paper describes a new technique that allows a permanent milk flow alongside an undisturbed healing process of all types of teat injuries.

Materials and Methods: Cows with covered or open teat injuries are suitable to benefit from the permanent milk flow system. A silicon-like catheter (TEATflow) has been designed for that purpose. The described surgery of open lesions as well differs from traditional methods as no blood stasis is applied. Prior to intervention Oxytocin (10 I.U.) is administered intravenously to achieve an intensive milk drop down. Single suture knots are carried out to achieve only a slight, but careful wound closure. This procedure is aimed to result in a faster consolidation of fibrocytes for a undisturbed healing process. To avoid any internal milk teat pressure, that might disturb the accumulation of fibrocytes, milk is allowed to permanently exit the teat by the inserted catheter for 9 consecutive days. The insertion is eased by application with the help of the traditional Fuerstenberg-catheter. Penethamat-Penicillin (2 x 5 Mio I.E./day i.m.) is administered systemically to reduce concurrent teat infections. No further local antibiotic treatment is applied. Nine individual cow case studies are reported. Occurrence of clinical mastitis, restoration of complete milkability and evolution of somatic cell count (prior and

around eight weeks after treatment) are documented as potential success criteria. The mean somatic cell count of all cows was 140.000/ml prior to treatment.

Results: All cows fully restored milkability. One cow was infected by a clinical mastitis, which reflects a mastitis infection risk of 11%. The mean somatic cell count (SCC) at eight weeks after surgery was 167.500/ml, which reflects a 19% increase of prior SCC. Due to the limited numbers this is not statistically significant. Six cows showed an augmented SCC, whereas 3 cows revealed a reduced SCC. All nine cows are listed below as follows: Cow No., race, type of teat lesion (external/internal), SCC prior and after event, Clinical mastitis/No clinical mastitis.

Cow No. 1: Holstein, external, 85.000/135.000, No clinical mastitis

Cow No. 2: Hostein, external, 140.000/170.000, No clinical mastitis

Cow No. 3: Simmenthal, internal, 60.000/105.000, No clinical mastitis

Cow No. 4: Simmenthal, external, 220.000/180.000, No clinical mastitis

Cow No. 5: Holstein, internal, 115.000/95.000, No clinical mastitis

Cow No. 6: Simmenthal, external, 240.000/330.000, Clinical mastitis

Cow No. 7: Simmenthal, external, 70.000/90.000, No clinical mastitis

Cow No. 8: Holstein, external, 240.000/280.000, No clinical mastitis

Cow No. 9: Simmenthal, external, 90.000/80.000, No clinical mastitis

Conclusions: The new treatment and surgery method for all type of teat lesions offers a very practical, simple and easy way to successfully regain milkability. As the risk for clinical mastitis seems lower than reported elsewhere, the permanent milk fow system obviously seems to allow a faster and full healing process at a very low level of infection risk due to the permanent open teat canal.

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The Use Of Bacteriophage In The Prevention Of Bovine Coliform Mastitis

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Objectives: The objectives of the current study were to determine if a broad host-range bacteriophage cocktail could be made against mastitis-causing *E. coli*, evaluate coliphage activity in raw milk, determine whether our cocktail could reduce adhesion and invasion of mammary epithelial cells by chronic coliform mastitis strains, and test whether a phage based intramammary ointment can inhibit bacterial growth.

Materials and Methods: Seventy-four bacteriophages were initially isolated using 36 clinical coliform mastitis isolates from dairies in Washington State. Each individual phage preparation was then tested for its ability to suppress growth of the 36 clinical isolates using spot lysis tests. Four phages were selected from this group as candidates for a cocktail, based on their distinct broad host ranges against these mastitis strains. Restriction fragment length polymorphism analysis was performed to determine whether each phage in the cocktail was distinct and phage genomes were subsequently sequenced. To assess the effects of different doses of bacteriophage cocktail on the growth of *E. coli* isolates from New York and Washington State, optical density growth curves were generated for each bacterial strain. Total bacterial counts in raw milk were measured after twelve hours of incubation with the phage cocktail using a range of initial bacterial inoculant doses of an *E. coli* mastitis strain. An immortalized bovine mammary alveolar epithelial cell line, MAC-T, was used for adhesion, invasion, and internalization assays

through a modified gentamycin protection assay. The bacteriophage cocktail was emulsified with a bismuth-based teat sealant and its activity tested in liquid growth media.

Results: The bacteriophage significantly inhibited growth of 58% of the Washington State isolates and 54% of *E. coli* mastitis isolates from New York State, suggesting that the cocktail of phages had a broad spectrum of action against relevant strains from two distinct geographies. The ability to suppress bacterial growth of these isolates in a liquid growth media was not affected by the ratio of bacteriophage particles to bacterial cells (multiplicity of infection, MOI) which were tested. For those *E. coli* that were completely inhibited by the phage cocktail, an MOI as low as 10 had the same effect as 10 µg/ml of ceftiofur on the growth rate of *E. coli* over a twelve hour period using optical density measurements. Previous work with *Staphylococcus aureus* and phage K has shown that bacteriophage activity is inhibited in raw milk; however a 3.3-5.6 log reduction of *E. coli* growth was achieved when co-incubated with our phage cocktail in raw milk over a twelve hour period at physiologic temperature. Tissue culture plates pre-treated with approximately 10⁸ phage forming units (PFU) of our cocktail reduced mammary epithelial cell adhesion of three different chronic mastitis *E. coli* strains by 98.0, 99.6, and 99.7 percent. Intracellular invasion of mammary epithelial cells, as measured by the gentamicin protection assay, was reduced for the three strains by 88.3, 98.1, and 74.3 percent. When combined with a bismuth-based teat sealant, the phage cocktail was able to inhibit bacterial growth when challenged with 1.6 x 10³ CFU/mL of a clinical mastitis *E. coli* strain.

Conclusions: This in vitro study shows that a cocktail of bacteriophage can inhibit growth of common clinical coliform isolates and is active in raw milk. Bacteriophage can potentially prevent chronic *E. coli* infections by reducing adhesion and invasion of mammary epithelial cells and can be combined with a teat sealant as part of a dry cow treatment. Results suggest that a bacteriophage-based dry cow treatment could reduce the incidence of coliform mastitis in dairy cattle; however it must first be shown that a specific dose of bacteriophage can protect cows from experimentally induced *E. coli* mastitis without inducing an inflammatory reaction.

Udder Health & Milk Quality

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Impact Of The Use Of A Mastitis Multivalent Vaccine: A Field Study.

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Objectives: The coliform mastitis causes severe losses on dairy farms. Traditional strategies based on prophylaxis and control programs are not sufficient in some cases. New tools have emerged in order to increase the immunity of animals with emphasis on vaccines for mastitis. The aim of this study was to determine the effects on milk quality, on the infectious status of cows and the use of systemic antimicrobial therapy after the use of a mastitis multivalent vaccine (STARTVAC®, Hipra Laboratories SA, Spain) in a farm with serious problems of coliform mastitis.

Materials and Methods: The farm had on average 33 milking cows with excellent milking routines and good hygiene and management practices. The results of bacteriological samples of clinical and sub-clinical mastitis, in the year preceding the first application of the vaccine showed greater proportion of *Escherichia coli* (56%), *Streptococcus* spp. (17%) and Coagulase Negative *Staphylococcus* (CNS) (10%); the therapeutic

protocols used were expensive and with little effectiveness.

The vaccination program was initiated in August of 2012, with the three-week interval between the first and the second application, and repeated every three months, in lactating and dry cows and heifers.

Results: The number of treatments with systemic antibiotic therapy was 46% (6) in the previous year of vaccination and in the first year post vaccination was 11% (2); in the second year no systemic antimicrobial therapy was used in mastitis treatment. It was observed a reduction in annual average of somatic cell count (SCC) in the bulk milk, from 341.000 cells per milliliter (cells/ml) in the period 2011/2012, to 266.700 in the first year (2012/2013) and 202.000 in second year of vaccination (2013/2014).

A significant reduction in the percentage of chronic animal and new infections was observed, especially in the second year after immunization. However, the farm still had a risk period in winter, linked to climatic factors.

Conclusions: The results of the introduction of a multivalent vaccine for mastitis show a reduction of SCC in the bulk milk tank, a reduction in the number of new infections and chronic animal and a significant reduction of the antibiotic systemic therapy. The benefits obtained with the vaccination program justify their use and maintenance on the farm and must be considered on mastitis control programs.

Udder Health & Milk Quality

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Dry cow therapy on organic farms – Selection criteria

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Objectives: Antibiotic dry cow therapy (DCT) is a key component of dry cow management. According to the guidelines of organic farming the use of DCT is restricted and selective DCT is applied. Due to low cure and a high new infection rates the mastitis prevalence at calving is high on organic farms. To improve the udder health situation in the dry period and for successful selective DCT an accurate selection method is required to correctly identify infected animals. The objective of this study was to find cow level factors associated with intramammary infections (IMI) at dry-off and use the results to create a decision tree for dry cows.

Materials and Methods: The field study was carried out on five organic farms with a mean of 135 ± 35 German Holstein dairy cows located in Lower Saxony, Germany. 250 cows that were dried of between June and October 2014 were recruited to the study. No attempt was made to influence the dry cow management in the trial farms. Dry cow therapy was administered by the farm owners. Individual cows information was obtained from data of the dairy herd improvement (DHI) test. At dry-off, all cows had a California Mastitis Test (CMT) performed and udder shape, teat condition, cow cleanliness, body condition score and milk yield were recorded. Quarter foremilk samples were collected and cultured at dry-off and two times after calving. Until 100 d in milk clinical mastitis data of the cows were documented. Infection status at dry-off was used as the dependent variable in a generalised estimating equation (GEE) model.

Results: In 39.5% of quarters at dry-off and in 71.5% of quarters in the post partum control no microbiological growth could be detected. The most frequent isolated pathogens were coryneforms (23.4% dry off/ 11.0% post partum) and coagulase-negative staphylococci (16.7% dry

off/ 12.6% post partum). The new infection rate on quarter level was 17.6%, the cure rate was 81.3%. The GEE proved that a geometric mean of the somatic cell counts (SCC) > 100.000 cells/ml in the last three DHI records is significantly associated with an IMI at dry-off (OR: 15.425, 95% CI: 5.670 – 41.930, P<0.001).

Conclusions: The selection of infected animals at dry-off is the key to successful selective DCT. The present study showed that the geometric mean of the SCC in the last three DHI records can be used as an indicator for having an IMI at dry-off (Figure 1).

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The cell wall component lipoteichoic acid of Staphylococcus aureus induces chemokine gene expression in bovine mammary epithelial cells

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Objectives: Staphylococcus aureus is a major cause of bovine mastitis, but its pathogenic mechanism remains poorly understood. Among the virulence factors of S. aureus, the cell wall component lipoteichoic acid (LTA) has been shown to play a pathogenic role in infectious diseases through its involvement in biofilm formation, which promotes bacterial adherence to the host. The objective of this study was to evaluate the role of LTA in the immune or inflammatory response of S. aureus-induced mastitis.

Materials and Methods: To reveal whether LTA of S. aureus could induce immune or inflammatory response mediators in mastitis, we determined the gene expression profile of bovine mammary epithelial cells (BMECs) stimulated with purified LTA alone or formalin-killed S. aureus (FKSA) constituents containing all bacterial components, including LTA, using cap analysis of gene expression (CAGE). First, we analyzed differentially expressed genes (DEGs) in the profile of BMECs stimulated with LTA alone according to the CAGE results. Next, we analyzed the DEG profile in the BMECs stimulated with FKSA according to the CAGE results.

Results: A total of 59,441 transcriptional start sites (TSSs) of DEGs were induced by the LTA stimulation, including 41 up-regulated and 141 down-regulated genes. On the other hand, a total of 57,417 TSSs of DEGs were induced by the FKSA stimulation, including 54 up-regulated genes and 15 down-regulated genes. Seven common DEGs (IL-8, CXCL6, MX2, CCL2, IL-1α, NFKBIZ, and PTGS2) related to immune or inflammatory mediators were up-regulated under both LTA and FKSA stimulation. Three of these genes encode chemokines (IL-8, CXCL6, and CCL2) functioning as chemoattractant molecules for neutrophils and macrophages.

Conclusions: Using CAGE, we were able to clarify the indeterminate expression of chemokine genes that have not been previously identified under induction conditions mimicking bovine mastitis. The simultaneous detection of temporal expression patterns of pathogens and host cells, especially during S. aureus mastitis, will help to define the pathogenesis of mastitis and provide further insight into

the molecular cross-talk between pathogens and host cells. The CAGE results described herein have revealed novel mechanisms of chemokine induction by *S. aureus* LTA.

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Effects of antibiotic dry cow therapy and internal teat sealant on milk somatic cell counts, clinical, and subclinical mastitis in early lactation

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Objectives: The objective of this study was to determine the efficacy of an internal teat sealant (TS; Teatseal; Zoetis Australia, Silverwater, NSW, Australia), when used in combination with antibiotic dry cow therapy (ADCT) administered at dry-off, on milk individual cow cell count (ICCC), milk production and components, and the incidence of clinical and subclinical mastitis in cows up to 60 days after calving, when compared to ADCT only.

Materials and Methods: Multiparous Holstein, Jersey, or Holstein cross cows (n = 2200) from 8 farms in Southern and Eastern Australia were randomly assigned to treatment of all 4 quarters with an ADCT alone or with ADCT and a TS (ADCT + TS) at dry-off in this randomized multi-site clinical trial. Milk yield, fat and protein percentage, and ICCC were measured at 14 ± 3 day intervals after calving for the first 60 days of lactation. The first measurement occurred between 10 to 24 days after calving. Clinical mastitis and health events were recorded from dry-off to 60 days in milk. Milk samples were collected from first cases of clinical mastitis and subjected to bacteriology. Log-transformed ICCC, ICCC weighted by milk yield, milk yield, and fat and protein percentage were analysed using a general linear mixed model for repeated measures, with the fixed effects of treatment group, time point, and the interaction of these effects, the random effects of farm, block, and animal, and the interaction terms for treatment group and farm, and treatment group, time point, and farm. The time to clinical mastitis (days post-calving) was analysed using survival analysis methods for censored data, with non-mastitis cases censored at the time of the last herd test up to 60 days post-calving. The presence or absence of subclinical mastitis post-calving (at least one ICCC of ≥ 250,000 cells/mL between 10 to 60 days post-calving) was analysed using a generalized linear mixed model with the fixed effect of treatment group, the random effects of farm and block, and the interaction term for treatment group and farm.

Results: Milk yield, ICCC weighted by milk yield, and fat and protein percentage were not affected by the interaction of treatment and time or treatment. Treatment with ADCT + TS at dry-off decreased the geometric mean ICCC by 11,500 cells/mL in the first 60 days of lactation, compared to use of ADCT alone (P = 0.021). Similarly, the geometric mean of the ADCT only group was 35% higher (95% CI: 5 to 72%) than that of the ADCT + TS group. The odds of at least one case of subclinical mastitis (ICCC ≥ 250,000 cells/mL) were 1.9 times higher (95% CI: 1.4 to 2.6%) with ADCT alone in the first 60 days of lactation, compared to the use of ADCT + TS. Use of ADCT + TS reduced the estimated incidence of at least one case of subclinical mastitis on all 8 farms, compared to use of ADCT alone. Only 4 cows that calved 40 to 100 days after dry-off had a first case of clinical mastitis in the dry period. Five percent of cows that calved 40 to 100 days after dry-off had a first case of clinical mastitis between 0 and 60 days in milk. Forty-three cases (5.7%) occurred in the ADCT group and 33 (4.3%) for the ADCT + TS group out of 1528

cows included in this analysis but this was not significantly different (P = 0.194). Proportional hazards estimates of survival showed there was no difference in the number of days post-calving to detection of first cases of clinical mastitis between the ADCT and ADCT + TS groups over the first 60 days postpartum (P = 0.153). The estimated hazard ratio for clinical mastitis over this period in the ADCT + TS cows was 0.70 (95% CI: 0.43 to 1.14%).

Conclusions: The combination of ADCT and TS provides benefits over ADCT use alone through improved prevention of the incidence of subclinical mastitis and reduced ICCC in the first 60 days of lactation. The prevention of mastitis is dependent on many factors in addition to dry-cow therapy, including aseptic dry-off technique, environmental and milking hygiene, and monitoring and management of cows, particularly during high risk periods. Adoption of the use of the combination of ADCT and TS may be beneficial for reduction of ICCC.

Udder Health & Milk Quality

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Development Of New Intramammary Mastitis Treatment Containing Lasalocid

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Objectives: The objectives of this study were to test the potential of re-purposing ionophores for treatment of bovine mastitis using lasalocid as a model drug. The study aims were to improve the pharmacokinetic characteristics of the lasalocid using the solid dispersion delivery system, test its antimicrobial activity in vitro and preliminary cow-level assessment of the safety, preliminary withholding period and efficacy against mastitis using *Streptococcus uberis* challenge model. Lasalocid, a polyether ionophore has favourable for mastitis treatment due to the lack of clinically significant resistance and transmissible resistance elements.

Materials and Methods: Minimum inhibitory concentrations (MIC) were determined using the broth microdilution technique. Bacterial isolates were obtained from clinical cases of bovine mastitis occurring in Australian dairy cattle. In total, 57 gram-positive mastitis isolates were tested (*Streptococcus agalactiae* (12), *Staphylococcus aureus* (20), coagulase-negative staphylococci (14) and *Streptococcus uberis* (11)) plus 10 isolates of methicillin-resistant *Staphylococcus aureus* obtained from cases of infection in small animals.

The development of formulation followed few steps, namely determination of the solubility of lasalocid, preparation of the SD delivery system, and characterisation of the SD. The size reduction of the lasalocid alone and the SDs to micro-metre grade or to nano-metre grade was carried out by using sieves or a ball-mill and homogenizer. The tested formulations contained 150, 300, 400, 600 or 900 mg of the active.

For the cow-level experiments cows were in various stages of an active lactation. They were aged 2-10 years and four functional quarters, had no significant teat lesions, were in good health, had known somatic cell count history of less than 200,000 cells/mL and had not received antimicrobial or anti-inflammatory therapy within 14 days of the study commencement. For the safety and preliminary withholding period each

quarter of each cow was treated with the same dose of the formulation (e.g. 4 intramammary tubes per cow). For estimation of the efficacy only quarters that were diagnosed with mastitis were treated with the test or the reference product.

Results: Lasalocid demonstrated antimicrobial activity against the major Gram-positive mastitis pathogens including MRSA (MIC₅₀ range 0.5–2.0 µg/mL and MIC₉₀ of 0.5-4.0 µg/mL). However, lasalocid was not tested against Gram-negative pathogens capable of causing mastitis. In other work carried by this group we have demonstrated lack of efficacy of lasalocid against gram-negative pathogens.

The solubility test confirmed limited, ion-strength-dependent water solubility of lasalocid. Lasalocid has a low solubility in water but a significantly higher solubility in organic solvents such as DMSO and methanol.

The safety of the formulations differed between formulations, the size of the particles and the total dose of the active. A transient elevation of the somatic cell count after intramammary administration of any product was observed. The formulations that contained the largest dose of the lasalocid (900mg in all 4 quarters) caused mild depression and decreased appetite. The formulation containing Soluplus® was associated with a significant rise in the somatic cells and other clinical observation. The PVP-lasalocid-SD exhibited a faster distribution of active in milk than either nano- or micro-sized lasalocid estimated WHP of 5-6 milking.

All challenged quarters developed mastitis. The cure rates after the intramammary administration of 6 tubes in 3 days containing 150 or 300 mg PVP-lasalocid-SD provided effective treatment with a higher mastitis cure rate (66.7-100.0%) compared to the treatment with 3 tubes in 3 days of the reference cloxacillin-containing product (66.7%).

Conclusions: The results from this study indicate that polyether ionophores, using lasalocid as a model drug, can be successfully repurposed for treatment of bovine mastitis. The in vitro antimicrobial spectrum of lasalocid indicated that it can be used for treatment of bovine mastitis caused by the common gram-positive mastitis pathogens. The pharmacokinetic characteristics of the lasalocid using the SD delivery system study were improved. The PVP-lasalocid-SD demonstrated the best distribution and maintenance of the therapeutic concentrations and was safe. The preliminary withholding period of the PVP-lasalocid-SD formulation was acceptable.

Udder Health & Milk Quality

P01-001-186

Monensin continuous-release intra-ruminal device: effects on milk quality and ripened Parmigiano Reggiano cheese

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Objectives: The objective of this study was to determine the influence of monensin continuous-release intra-ruminal device (Kexxtone®) used to reduce the incidence of ketosis in fresh dairy cows, on milk and cheese quality. Because Parmigiano Reggiano is an Italian DOP (Protected Designation of Origin) raw milk cheese, without additives and with a long ripening period, it should easily highlight any possible negative effect of the treatment on the component quality of the final product.

Materials and Methods: The device was administered to 33 cows, 21 days before parturition as label indications. After calving, cows that received the bolus became part of a treated group (T) while a control group (C) was set up with untreated cows. The groups were housed in two pens, fed the same total mixed ration and milked twice a day. Milk and lactoserum produced by the groups were kept separated and each group produced 2 cheese wheels (45 kg each) per day. During the 20 experimental weeks, morning tank milk and lactoserum were sampled twice a week, for a total amount of 35 samples per group. Group milk was analyzed for fat, casein, protein, lactose, titratable acidity (°SH), pH, clotting characteristics (LDG), Somatic Cell Count (SCC), urea and total bacterial count (TBC). Lactoserum was analyzed for titratable acidity, fermentative activities at 45, 52, 54°C (ΔSH) and total lactic acid bacteria count (UFC/ml).

All 552 cheeses produced during the trial (276/group) were examined at 6 months of ripening through x-ray analysis. An official expert of the Consortium of Parmigiano Reggiano evaluated the cheeses after 12 months from production, in order to highlight any defects. After 18 months of ripening, 48 cheeses (24/group) were analyzed by an expert panel in order to assess any sensorial differences among groups. The weight of every cheese was recorded after 36 hours and 18 months from production to determine the final cheese yield (kg cheese/100 kg milk).

Data were analyzed using one-way ANOVA with Tukey test for mean comparison (SPSS 17.0, USA). Statistical significance was fixed for p<0.05.

Results: Milk and lactoserum maintained the same quality in both groups with few significant differences: SCC was significantly lower in the treated versus the control group (135.3±34.6 vs 214.80±46.1, *1000/ml, p<0.01) and casein and fat yield (kg/cow/day) was higher for T than C group (0.92±0.04 and 1.29±0.11 vs 0.80±0.04 and 1.09±0.07, p<0.01). Daily individual cheese yield (kg cheese/cow/day) was 0.4 kg higher for T versus C cows (2.89±0.1 vs 2.42±0.1, p<0.01).

A significantly higher percentage of T cheeses showed absence of defects at x-ray analysis (93.8 vs 87.7 %, p<0.01) as well as at the Consortium's evaluation (98.5 vs 93.5 %, p<0.01).

Organoleptic analysis showed an overall comparable sensorial profile between cheeses with few significant differences. T samples showed a slightly slower ripening process indicated by higher intensity of butter and sweet aroma (3.2±0.4 and 3.5±0.3 vs 3.0±0.4 and 3.4±0.4, p<0.05) and lower rind and spicy flavours (2.0±0.4 and 1.8±0.4 vs 2.1±0.4 1.9±0.4, p<0.05). In addition, T cheeses had less intense, negative aromas, such as pungent, acetic and "stall", than C cheeses (2.1±0.4 vs 2.2±0.4, p<0.05).

Conclusions: These results suggest that treatment with a monensin continuous-release intraruminal device (Kexxtone®) in cows during dry off, doesn't affect milk, lactoserum or final cheese quality.

In addition, it showed a positive effect on somatic cell count and a good influence on ripened cheese. These positive implications could be associated with a general improvement of fresh cows' health status.

Udder Health & Milk Quality

P01-001-187

Effect of coffee ground silage in reducing somatic cell count in bovine subclinical mastitic milk

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Objectives: Coffee grounds are a rich source of the antioxidant polyphenol, and so are known to have beneficial effects on the immune system as an oxygen scavenger. The present study investigated how the silaging of coffee grounds improved the palatability of cattle fodder, increased the antioxidant activity of cow milk, and altered immune cell kinetics, thereby reducing the somatic cell count in milk.

Materials and Methods:

Coffee ground silage

The coffee ground silage converted into feed for dairy cows using a new lactic acid fermentation technique was made with coffee grounds supplied from Starbucks stores in Japan.

Measurement of milk somatic cell count

Measurement was conducted on 44 quarters from 20 cows with subclinical mastitis. Eleven of the cows (22 quarters) received 1.5 kg/day of coffee ground silage and the remaining 9 cows (22 quarters) were assigned to the control group with no coffee ground silage. Milk samples were collected before and 1, 2, 3, and 4 weeks after introducing the feed, and somatic cell count in milk was measured using a DeLaval cell counter.

Measurement of antioxidant activity

Measurement was conducted on 11 cows with subclinical mastitis bred. Five of the cows received coffee ground silage and the remaining 6 cows were assigned to the control group with no coffee ground silage. Blood and milk samples were collected before and 1, 2, 3, 4, and 5 weeks after introducing the feed, antioxidant activity in the form of oxygen radical absorbance capacity (ORAC) was measured in blood and milk, and lipid peroxidation in the form of thiobarbituric acid reactive substances (TBARS) was measured in blood.

Milk immune cell kinetics

Testing was conducted on 12 cows with subclinical mastitis bred. Seven of the cows received coffee ground silage and the remaining 5 cows were assigned to the control group with no coffee ground silage. Milk samples were collected before and 1, 4, and 7 days after introducing the feed, and leukocyte populations in milk were analyzed using flow cytometry.

Results: Unlike the control group, which showed no significant decrease in somatic cell count in milk, the cell count at Week 5 in the coffee ground silage group had decreased significantly from baseline ($p < 0.05$). Blood and milk ORAC values were also significantly higher at Weeks 3, 4, and 5 in the coffee ground silage group compared to baseline levels ($p < 0.05$), and blood TBARS values at Week 5 were significantly lower in the coffee grounds silage group than in the control group ($p < 0.01$). Analysis of milk leukocyte populations up to Day 7 revealed a significant increase in CD4+ cells and CD14+ MHC class II+ cells at Day 1 versus at baseline in the coffee ground silage group ($p < 0.05$).

Conclusions: The study findings show that supplying coffee ground silage to dairy cows increases antioxidant activity in blood and milk and decreases the somatic cell count. Findings for immune kinetics up to Day 7 also suggested that coffee ground silage has an effect on the immune system.

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P01-001-188

Observation of Mastitis Parameters in farms using a Polyvalent Mastitis Vaccine. An update after 3 Years

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Objectives: Monitoring the dynamic nature and magnitude of the mastitis challenge, prevalence and incidence within the modern dairy herd is becoming common place around the developed dairy industries of the world. The iterative process of regular interpretation of prevalence data (Individual Cow Somatic Cell Counts [ISCC]), incidence data (Clinical mastitis records) and how these data change over time both within lactation and over the dry period can give much information about the transmission patterns and likely origin and duration of intramammary infections within a dairy herd.

Materials and Methods: The initial aim of this project was to apply these diagnostic and monitoring techniques to three herds chosen for a high prevalence of *Staphylococcus aureus* over the 12 months prior to and during the 12 months of a rolling 3 month vaccination program with a polyvalent mastitis vaccine (Startvac, Hipra). After this initial observational period two of the herds decide to continue with the vaccination program.

All cows received 2 vaccine doses 3 weeks apart followed by a rolling policy of quarterly boosters. In calf heifers were batched to receive 2 doses 3 weeks apart with the second dose no less than 10 days prior to expected parturition. Heifers then joined the rolling 3 month booster program.

Data is compared at 6 month intervals from 12 months prior to and 36 months after initiating a whole herd vaccination policy.

Results: First 12 months (3 farms vaccinating)

The parameters observed were:

- *S. aureus* prevalence - using individual cow bacteriological culture of all cows in milk before and after the project.
- Clinical mastitis incidence.
- Reduction in clinical mastitis incidence in all 3 herds by 54%
- Routine monthly individual cow SCC data – using a 200,000 cells per ml threshold to indicate infection status.
- Reduction in proportion of infected cows in the herd (% cows > 200,000 cells per ml) by 4%
- Reduction in First infections in the 3 herds (high 1st cell count after parturition) by 71%. This effect was seen in both cows and heifers
- Reduction in New infections in two herds and increase in one herd (1st high SCC in current lactation but not 1st SCC after parturition)
- An estimation of cost benefit for the 12 month period of vaccination for the 3 herds was six times the cost of the vaccine.

From 12 to 36 months (2 farms vaccinating)

- Clinical mastitis incidence: both herds had an increase in clinical mastitis in the 12 to 18 months period after initiating vaccination. They had a likely lactation environmental outbreak of clinical mastitis. But they have subsequently returned to the improved mastitis rates seen subsequent to vaccination.
- The reduction seen in the first 12 months after vaccination for the proportion of infected cows and First infections was maintained in the next 24 months.

Conclusions: In this longitudinal study during three years of using a polyvalent mastitis vaccine despite some short lived fluctuations an improvement in the clinical mastitis and ICSCC was observed. It is important to remark that better results are obtained after 6 months using the rolling protocol - in common with other studies as the improvements take time to accumulate. Vaccination is a valuable tool to control *S. aureus* infections but it is important to remember that as with any control tool, vaccination is not a panacea and consistent good mastitis management is essential.

Udder Health & Milk Quality

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Milking-time testing in herds using Automatic Milking Systems

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Objectives: The aim of the study is to describe the variation of common milking-time test parameters for defined teat sizes and milk flow rates. Based on these results, we will simulate different milking-time test strategies to establish scientifically based milking-time test routines in AMS herds. In addition we aim to determine reference values for milking time test.

Materials and Methods: Milking-time tests on all teats on all cows (n=100) milked by two DeLaval VMS's at the Farm Animal Research Centre at Norwegian University of Life Sciences will be performed. The milking-time test will be performed using VaDia vacuum logging devices. Teat size, shape and condition will be registered before and after milking. Milk flow data will be collected from the milk meters. VaDia Suite data analysis tool will be used to calculate average milk flow, machine on-time, average vacuum level for the whole milking, during the peak-flow period and during overmilking, mouthpiece chamber vacuum during peak-flow period and number of vacuum fluctuations (www.biocontrol.no). Variance estimates for milking-time test parameters for the herd and for groups of cows will be included in a simulation model to determine the number of cow measurements that are needed to make inferences for the whole herd.

Data collection is ongoing, and the analysis will be conducted in February 2016.

In addition, a large number of results from milking-time tests performed on Norwegian farms by TINE Advisory service will be combined with udder health- and milk quality results from the Norwegian Dairy Herd Recording System to establish reference values for milking-time test parameters as mentioned above.

Results: The Milking-time test results will form the basis for a decision tool, used by Dairy advisers, to give advice regarding vacuum levels, teatcup liner and other milking machine settings. By including the whole herd, a description of in-herd variation and a simulation of selecting a smaller number of teats for testing is calculated. First, we will present herd mean and variation for different milking-time test parameters, such as short milk tube vacuum during peak flow, mouthpiece chamber vacuum, average milk flow and machine on-time, and we will present how these parameters vary between groups of cows with different teat sizes. Second, we will present how selection of different number/proportion of cows affects the herd level conclusions. Third, we will present relations

between milking-time test results and herd health and milk quality results to suggest optimum vacuum levels in different parts of the teatcup during milking.

Conclusions: As a conclusion, we hope to suggest a minimum number/proportion of cows to be included in a milking-time test to be able to give high-quality advice on herd level. We also hope to draw conclusions on the relationship between teat size and milking-time test results. This will form the basis for further research on the topic. In addition we hope to suggest reference values for milking time test.

Comments: Data collection is ongoing, and the analysis will be conducted in February 2016.

Udder Health & Milk Quality

P01-001-190

Bacterial carryover and contamination in composite milk samples from automatic milking systems

Gunnar Dalen^{1,2}; Håvard Nørstebo^{1,2}; Anne Cathrine Whist²; Liv Synnøve Sølverød²; Olav Reksen¹ and New approaches for management and breeding of dairy cows, in automatic milking systems – The AMS project

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Objectives: The principle method in Norway for collecting milk samples from dairy cows for bacteriological analysis is by aseptic sampling from all four teats separately. Since the introduction of PCR tests for udder pathogens, an increasing number of farmers with automatic milking systems (AMS) have started to use composite milk as sample material for bacteriological analysis of mastitis pathogens. Our objective is to see whether analysis of composite samples correctly predicts the presence of bacteria in the udder compared to aseptic sampling at quarter level.

Materials and Methods: We will collect composite milk samples for bacteriological analysis from cows in the research facilities at the Norwegian University of Life Sciences (SHF). SHF has two lactation pens with one DeLaval Voluntary milking system station (VMS) in each pen. Commercial automated samplers (DeLaval) will collect the composite milk samples. To minimize carryover, DeLaval has optimized the two VMS at SHF. Aseptic teat sampling will be manual.

100 cows will be sampled in both the AMS and manually at the same day. The samples will be frozen and shipped to the TINE Mastitis laboratory in Molde. Sample analysis will be with both bacteriological culturing and identification with the MALDI-TOF technology as well as with a PCR test kit for udder pathogens.

Results: Other studies have shown carryover from cow to cow in composite samples taken in both AMS (Løvendahl 2005) and conventional systems (Mahmmod 2014), both for milk components and bacteria. We expect to observe a level of bacterial carryover in composite samples collected via the two AMS in our setup. We will quantify this carryover and analyze the diagnostic value of testing composite milk samples instead of aseptic teat samples. This includes statistical analysis of variation in bacteriological diagnosis and the difference in diagnostic accuracy between composite milk samples on udder level and aseptic teat samples at quarter level.

Conclusions: We expect bacterial carryover from cow to cow in samples collected via the AMS. We also expect there to be environmental contamination of the composite milk samples and a lower level of detail from the analysis of composite milk than from the analysis of aseptic individual teat samples. It is imperative that diagnostic tests reflect the actual situation. If the diagnostic value of composite samples is too low to be of use, this is an important finding for the farmers sampling milk via the AMS.

Comments: Samples will be collected and analyzed in January 2016.

Udder Health & Milk Quality

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Case study: Calculation of return on investment (ROI) after one year using a mastitis vaccination programme

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Objectives: To determine whether a mastitis vaccination programme meets its objectives in a dairy farm from the North of Spain, and to identify the benefits obtained on the basis of the calculation of return on investment (ROI).

Materials and Methods: A dairy farm with some 850 highly productive adult cows in the North of Spain, where the farmer presented us with the challenge of identifying whether the investment made in the implementation of a mastitis vaccination programme had been profitable. This was carried out after the consolidation of the vaccination programme and we present it below as an example and additional task of the work of the clinical veterinarian

In December 2013 a prophylactic vaccination programme of the whole adult herd was started with a mastitis vaccine (STARTVAC®, HIPRA). This decision was not taken as a therapeutic measure, which was not necessary, but as a farm excellence policy action and the pursuit of better technical and economic results based on the arguments put forward by the specialists (work organization, fewer cases of mastitis, less serious cases, better milk quality etc.).

Results: Technical results for 2013 (without mastitis vaccination) and 2014 (with vaccination) are presented in Table 1

Table 1: Main technical results of the implementation of the vaccination programme for 2013 (without mastitis vaccination) and 2014 (with vaccination)

Average number of cows (No.)	860	858
Mean monthly somatic cell count (cells/ml)	301	265
Linear Score (LS) based on individual SCC	3.04	2.66
Cases of mastitis (CM) (No. of cases per year)	505	400
Monthly mastitis	4.9 %	3.8 %
Standardized daily production (L/lact.cow /day)	34.40	35.12
Annual production (L/cow/year)	11,311	11,790
Quality premium (months with premium)	0	3
Quality premium (months with penalty)	7	2
Quality deduction (months with penalty)	0	0

Investment of 12,900 € in vaccination and its implementation facilitated lower costs associated with the treatment of clinical mastitis (-14,199 €), a higher milk value (+39,075 €) and a reduction in the SCC, which

contributed to the improvement in production (+11,269 €). The greatest return on this farm was the increase in quality premiums (61%) followed by the costs associated with mastitis (22%) and the reduction in SCC (17%). The ROI obtained after the investment was 500 % (64543/12900 x 100).

Conclusions: The ROI obtained after the investment was 500 %. On farms which do not have a quality premium, the ROI will be lower, but clearly positive at 197 % (25468/12900 x 100). The recommendation is to continue with the established vaccination programme with a reassessment next year.

Udder Health & Milk Quality

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Case Study: Use Of Vaccination To Control Bovine Mastitis Caused By Klebsiella

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Objectives: Mastitis is an important animal disease causing decreased production and profits on dairy farms. J5 vaccines against gram negative pathogens have been used in the US for over 40 years. Since 2009 a polyvalent vaccine protecting against coliforms, Staphylococcus aureus and coagulase negative staphylococci (CNS) has been available in Europe (STARTVAC®).

The objective of this field trial is to find out on a farm with known Klebsiella mastitis problems if vaccinated cows become chronic after a clinical or subclinical infection with Klebsiella.

Materials and Methods: In a herd with 110 cows in milk, located in Flevoland in The Netherlands, during 2011 a problem with Klebsiella was diagnosed. Cows are housed in freestalls with dried manure solids as bedding material. Cows are milked in 2 robots (Lely A4) 3-3,2 times a day. After milking, a lactic acid product is applied as a spray.

All cows have been vaccinated since December 2014 including the heifers starting from four months before calving. Vaccinated animals received the first two doses in a four weeks interval. After this, a blanket vaccination protocol was done (single dose applied every three months for all cows and heifers). Animals with identified clinical mastitis were sampled in a sterile way before treatment, then a PCR test of the milk sample was performed

Every 3-4 months all cows with an elevated somatic cell count in two consecutive tests (cows >250.000 and heifers >150.000) were sampled from all quarters. Later samples were pooled on cow level and a PCR was performed.

Cows with a positive Klebsiella result on both a clinical or subclinical mastitis are followed in time in order to check the presence of Klebsiella.

Clinical mastitis cases are being treated with a combination of cefalexine and kanamycine and injected with a combination of trimethoprim and sulfadoxine. Subclinical mastitis cases are not treated.

Results: In 2014 there have been 31 cases of clinical mastitis. 15 cases were severe. Milk samples have been taken from 11 of these severe cases. Results were; 1 E. coli and 7 Klebsiella cases. Of these 7 Klebsiella cases 5 cows have either died or have been euthanized.

In 2015 there have been 17 cases of clinical mastitis. After sampling all of them, in 4 cases Klebsiella was found. From the subclinical mastitis cases (2015), in 4 cows Klebsiella has been found. Non of these Klebsiella cows have died or have been euthanized.

During resampling, 6 out of the 8 cows (75%), that had been positive for *Klebsiella* didn't show any *Klebsiella* anymore. In 2 cases (25%) *Klebsiella* was found in one or more samples during the resampling.

The "Defined Daily Dose Animal"(DDDA) for mastitis injectors from 1-1-2014 to 1-10-2014 was 1,07 and from 1-1-2015 to 1-10-2015 it is 0,66. The(total) DDDA for this farm from 1-1-2014 to 15-11-2014 was 4,45 and from 1-1-2015 to 15-11-2015 it is 3,29.

Conclusions: On a farm with known *Klebsiella* mastitis problems vaccination can help controlling disease by preventing quarters to become chronically infected. In addition cows suffer less severe clinical mastitis and cure rates have improved after vaccination.

However it is advised to track all confirmed *Klebsiella* mastitis cases. It is also important to sample high somatic cell cows on regular basis in order to quickly identify possible *Klebsiella* carriers.

Udder Health & Milk Quality

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Clinical efficacy of intramammary prednisolone treatment of naturally occurring clinical mastitis

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Objectives: Most EU dairy cows affected with clinical mastitis, are, in addition to antibiotics, intramammary treated with prednisolone. Experimental studies support prednisolone treatment showing a reduction of clinical signs, but in such studies many variations between animals are excluded and treatment occurs within a few hours after challenge. The questions then is whether these effects can be translated to the field. Peer reviewed field study papers supporting prednisolone treatment in mastitis are scarce. Therefore, the objective of this study was to determine the clinical efficacy of prednisolone in clinical mastitis cases under field conditions.

Materials and Methods: The trial was a multi-centered, double blinded, randomized, controlled and performed in France according to GCP guidelines. One hundred and twenty cows with naturally occurring mastitis in a single quarter were randomly (SAS[®] software generated list) allocated to two treatment groups. The first group (Group 1) was treated intramammary, with 300 mg of cefapirin and the other group (Group 2) received 300 mg cefapirin plus 20 mg of prednisolone via the same route. Treatment schedule for both groups was 2x per day, after each milking with a 12 hour interval. Clinical examinations (rectal temperature, general condition, udder firmness, udder induration, udder pain, and milk quality) were performed at each morning and evening milking from day 0 to day 5 with a predefined clinical scoring system (0= normal, 1= light, 2=moderate, 3= severe)). Bacteriological analysis was only performed on pre-treatment milk samples of the affected quarter. Primary clinical efficacy parameters were the evolution of clinical signs during and shortly after treatment (4 days after the last treatment). Clinical condition of a cow was considered normal if clinical signs of clinical mastitis (milk aspect, udder and general condition) were absent, and the rectal temperature was < 39.5°C). Only cows affected in 1 quarter were enrolled, therefore the cow and quarter analysis are identical.

Results: Finally, a total of 117 cases were included in the trial from which 4 were excluded post-admission. This resulted in 113 cases included, 55 in Group 1 and 58 in Group 2. Reasons for post-admission withdrawal

were mainly deviations from the protocol, i.e. mastitis in a second quarter (n = 23), off protocol treatment during the trial period (n = 1).

Although all study data are collected, statistical analysis is currently performed, using a multi-variable logistic regression model including risk factors for clinical effects of prednisolone. Results will be available soon and can be presented at the WBC in Dublin.

Conclusions: The authors emphasize this is a unique and therefore important study for field practitioners to scientifically justify routine prednisolone treatment of clinical mastitis in the field. However, the deadline of the WBC abstract submission is a too early to draw final conclusions at this moment. Conclusions will be available for presentation at the WBC congress in Dublin.

Udder Health & Milk Quality

P01-001-195

Effectiveness and safety of a novel flunixin meglumine transdermal pour-on solution in the treatment of bovine mastitis

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Objectives: Anti-inflammatory drugs are commonly used as adjunct therapy to antibiotics to treat clinical mastitis, since many of the physiological and pathological changes associated with clinical mastitis are a result of the inflammatory response to infection. Flunixin is a NSAID used for the relief of pain and control inflammation and pyrexia associated with diseases of different origin and nature. A 50 mg/mL flunixin transdermal formulation (Finadyne[®] Transdermal) is available to be used in cattle. The objective of the study was to demonstrate the safety and effectiveness of flunixin transdermal in the treatment of clinical mastitis in dairy cows.

Materials and Methods: A total of 133 dairy cows, showing severe signs of mastitis, were randomly assigned to treatment with either the test product, flunixin transdermal or the negative control product, a red dye saline solution (to preserve masking), both administered topically along the dorsal midline . All animals received intramammary antibiotics and systemic antibiotics starting at 6 hours post-treatment. The animals were observed for clinical signs of disease for 6 hours post-treatment and daily for 5 consecutive days.

Results: The decrease in rectal temperature 6 hours post-treatment was greater in the flunixin group (-1.8°C) compared to the control group (-1°C). This difference was statistically significant (p<0.0001) and the superiority of the flunixin transdermal to the control was confirmed.

The alleviation of pain, firmness and swelling of the udder was also significantly greater at 6 hours (p<0.0001) and at 24 hours (p<0.05) after treatment initiation in the flunixin group compared to the negative control group. Rectal temperature and clinical index (general attitude and udder clinical signs) improved over time.

Neither flunixin nor control had a negative influence on the health status, confirming that flunixin transdermal is safe.

Conclusions: The new 50 mg/mL flunixin transdermal demonstrated strong anti-pyretic effect and anti-inflammatory properties, providing a convenient and suitable adjunct therapy to anti-infectives used in cases of mastitis infections in cattle to reduce pyrexia and to alleviate clinical signs of pain, firmness and swelling of the udder.

Udder Health & Milk Quality

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Prevalence, Colony-Forming Units, And Antibiotics Sensitivity Of Klebsiella Pneumonia In Mild Cases Of Mastitis

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Objectives: Coliform is reportedly one of the common causative bacteria frequently isolated from >50 % of mild cases of mastitis, which secrete a variety of forms of denatured milk but have no systemic signs. However, the previous reports describing clinical correlation between simple intra-mammary infection of *Klebsiella pneumoniae* (K.p.) and occurrence of mild signs of mastitis have been rare. The purpose of the present study is to clarify the clinical implication of infection with K.p. associated with mild cases of mastitis from the prevalence, colony-forming units, and antibiotics sensitivity of K.p.

Materials and Methods: Specimens were 2075 milk samples of 855 mild cases of mastitis breeding in 37 farms in Tottori prefecture between October 2012 and March 2015, and 31 specimens obtained from cows with peracute mastitis. K.p. was isolated, and measured the bacterial counts in milk (\log_{10} CFU/ml) on MCIC agar under incubation at 37°C for 24 h, and identified using the API 10 test (BioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibilities of the isolates to 16 types of antimicrobial agents were determined by the disc diffusion method in accordance with the guidelines of the Clinical Laboratory Standards Institute. Prevalence and bacterial counts of K.p. in milk samples were statistically compared between months using a chi-squared test, and a one-way analysis of variance test. A milk sample, in which K.p. was isolated 5.0 \log_{10} CFU/ml, was defined as a high isolated milk (HM). The prevalence of HMs were compared between months with a chi-squared test. Prevalence of antimicrobial resistant K.p. strains for each 16 antimicrobial agents were compared between cows with mild mastitis and peracute mastitis, and between 4 farms, where >10 K.p. strains were isolated from 2012 to 2015, with a chi-squared test. A p value of < 0.05 was considered statistically significant.

Results: K.p. was totally isolated in 155 of 2075 milk samples. The monthly prevalence of K.p. ranged from 3.6 to 13.7 %, and was significantly ($p < 0.05$) higher in July (12.4 %), and September (13.7 %). Averages of the bacterial counts of K.p. in milk samples were beyond 5.0 \log_{10} CFU/ml between February and October, although there were no significant differences between months in the K.p. CFU/ml of milk. The monthly prevalence of HMs averaged 56.0 % (ranging from 0.0 to 83.3 %), and was significantly ($p < 0.05$) higher in Jun (83.3 %) than in other months. Mild cases of mastitis had a comparatively higher prevalence of antimicrobial resistant K.p. strains for 5 types of cephalosporin antibiotics (used on screening tests for ESBL-producing bacteria) than those in the cows with peracute mastitis: the prevalence in the cows with mild mastitis ($n = 155$) and peracute mastitis ($n = 31$) was 8.0 % and 4.0 % for ceftazidime, 16.6 % and 4.8 % for ceftriaxone (CTRX), 18.5 % and 4.8 % for cefotaxime (CTX), 11.9 % and 4.8 % for cefpodoxime, and 9.3 % and 4.8 % for aztreonam (AZT), respectively. Of 4 farms, a farm had a significant ($p < 0.05$) higher prevalence of antimicrobial resistant K.p. strains for CTRX (36.4 %), CTX (36.4 %), AZT (27.3 %), and minocycline (16.7 %) than the other farms (resistance rates of 0.0 to 14.8 % for CTRX, 0.0 to 16.4 % for CTX, 0.0 to 10.0 % for AZT, and 0.0 to 1.6 % for minocycline). Another farm had a significant ($p < 0.05$) higher prevalence of antimicrobial resistant K.p. strains for tetracycline (83.3 %) than the other farms (25.0 to 50.0 %).

Conclusions: The present data suggests the following factors for mild cases of mastitis due to intra-mammary infection of K.p.: 1) A high-temperature and humidity particular in Japan relates to increases in the prevalence and bacterial counts.; 2) Higher intra-mammary K.p. infection is not always absolutely required for determining the severity of mastitis.; 3) Development of antimicrobial resistant K.p. may correlate with difficulty in healing, and chronicity, following occurrence of mild mastitis.; 4) Differences between farms in the infection patterns of antimicrobial resistant K.p. may be due to inappropriate use of antibiotics on particular farms.

Comments: Intra-mammary infection of *Klebsiella pneumoniae* was commonly focused on occurrences of peracute mastitis. However, the present study indicates that intra-mammary infection of *Klebsiella pneumoniae* is one of the important factors for occurrences of mild mastitis by long-term bacterial examinations for a great many specimens. Especially, mild cases of mastitis caused by intra-mammary infections of K.p. predict to be an important problem on herd health, and public health, based on the higher intra-mammary infection rate of *Klebsiella pneumoniae*, and the higher prevalence of antimicrobial resistances.

Udder Health & Milk Quality

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Characteristics of mastitic mammary secretions from Holstein cows at 8–12 days before parturition

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Objectives: In dairy cows, the incidence of new intramammary infections increases around and during the dry period of a lactation cycle. An intramammary infection during the dry period is considered to cause mastitis in the early lactation stage, and incidence of crisis of clinical mastitis actually increases after calving. Diagnosis of mastitis before calving will lower the loss of milk production in the early lactation stage if measures are undertaken before the beginning of lactation. The objectives of this study were to evaluate the relationship between intramammary infection and elemental characteristics of mammary secretion at the late dry period.

Materials and Methods: Mammary secretions were collected from 24 quarters of 6 Holstein cows at approximately 10 days (8-12 days) before parturition; the cows were raised in local farms in Japan. No obvious clinical signs of inflammation (redness, swelling, heat or pain) were found on their surfaces of udders. The mammary secretions were subjected to microbiological cultivation tests for identifying mastitis causative pathogens and counting somatic cells by direct microscopic observation, following macroscopic observations such as viscosity, color, including contamination of the blood pigment or red blood cells, and presence of gelatinous fibrin, milk clots, or flakes. Viscosity was divided into 3 classes, glutinous starch syrup or colostrum-like, normal milk-like, and serous fluid-like, and mammary secretions that showed starch syrup or colostrum-like viscosities were determined as normal. Concentration of serum albumin was determined using single radial immunodiffusion after removing somatic cells, fat, and fibrin from the mammary secretions by centrifugation.

Results: Macroscopic abnormalities were observed in 16 of 24 mammary secretion samples, and abnormal viscosity was found in all the

samples, except samples in which ostensible viscosity was increased by gelatinous fibrin. Mastitis causative bacteria (environmental streptococci, coagulase-negative staphylococci or *Corynebacterium bovis*) were isolated from 14 samples, and some macroscopic abnormalities were found in all the samples. In all of the abnormal mammary secretions, somatic cell counts and serum albumin concentration were apparently increased, identifying the occurrence of mastitis in the mammary glands.

Conclusions: At approximate 10 days before parturition, an intramammary infection caused mastitis, and mammary secretions showed abnormal macroscopic findings. Infiltrations of somatic cells and serum albumin were found in the mammary secretions. Lower viscosity of the mastitic mammary secretion could be caused by dilution with exuded serous fluid. Diagnosis of mastitis before parturition may help in controlling mastitis during the periparturient period.

Udder Health & Milk Quality

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Early Lactation Extended Therapy Against *Staphylococcus Aureus* Intramammary Infections In Heifers: A Randomized Controlled Trial

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Objectives: Once firmly established, *S. aureus* intra-mammary infections (IMI) are very difficult to cure. We could hypothesize that higher cure rates could be obtained in infected first parity cows treated promptly during the early lactation period. The general goal of this study was to evaluate the effect of an extended therapy using pirlimycin at calving for first lactation cows infected by *S. aureus* and identified using Petrifilm™ Staph express (STX) plates. The specific objectives were to assess cure rates in the treatment group and to assess test characteristics of the STX when used to identify infected quarters in early lactating heifers.

Materials and Methods: Study design was a randomized controlled trial. All first lactation cows with 4 functional quarters coming from a convenient sample of 21 dairy herds in the Drummondville area (Qc, Canada) and calving between May 2014 and December 2015 were enrolled. Quarter milk samples were collected from heifers in the first 10 days after calving. Quarter milk samples were diluted 1:10 with sterile water and inoculated on STX plates which were placed in an incubator at 35°C for 24h. After 24h, each Petrifilm was read by a trained technician and, when colonies were detected, a confirmation disk (DNase) was used to confirm *S. aureus* diagnostic. All presumptive colonies of *S. aureus* identified on STX were confirmed as such in a reference laboratory using standard methods. Positive quarters were randomly allocated to the control or treatment group using a random number generator. Cows in the treatment group received an extended therapy using pirlimycin: the producer administered 1 intramammary tube containing 50mg of pirlimycin hydrochloride (Pirsue®) in each infected quarter, once a day for 8 consecutive days, using a sterile technique. The control group remained untreated. Milk samples of the infected quarters in both groups were taken on day 7, 14 and 21 following treatment allocation and sent frozen to the reference laboratory where they were analysed using standard methods. A bacteriological cure was defined by the absence of *S. aureus* in the 3 post-treatment milk samples.

Results: For these preliminary analyses, effect of treatment group on odds of cure of the IMI was computed as odds ratio (OR) and 95% confidence intervals (CI) using methods for 2x2 tables. Recruitment of first parity cows in the trial is still ongoing. Up to now, 472 heifers were sampled and a total of 68 were confirmed infected by *S. aureus* in the first 10 days after calving indicating a prevalence of *S.aureus* IMI of 14.4% (95% CI: 11.5, 17.9). Thirty-six infected heifers were randomly allocated to the treatment group and 32 were allocated to the control group. The observed cure rates so far were 67% (24/36) and 41% (13/32) in the treatment and the control group, respectively. Preliminary analyses indicated significantly higher odds of cure (OR: 2.9; 95% CI: 1.1, 7.9) in treated cows compared to the control group. Regarding accuracy of STX plate for identifying *S.aureus* infected quarters in early lactating first parity cows, 27% (25/93) of the positive STX were found to be false positives when compared to the bacteriological results from the reference laboratory.

Conclusions: Preliminary results show that a high cure rate can be achieved with the proposed extended treatment when instigated within two weeks of calving. An unexpected high self-cure rate was observed in the control group. Petrifilm STX can be used to detect *S. aureus* infected heifers early in lactation, but a substantial number of false positive results should be expected. The RCT will be completed and then fully analysed. Additional analyses will also be conducted to try to propose better guidelines for STX interpretation to reduce the proportion of false-positive in the future.

Udder Health & Milk Quality

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How farmers experience the introduction of selective dry cow therapy

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Objectives: In January 2013, selective dry cow therapy (SDCT) became obligatory in the Netherlands. Experimental studies were done on the effect of SDCT on udder health (Scherpenzeel et al., 2014). How SDCT was executed in practice, and how farmers experienced SDCT, was unknown, while it is important for quantifying the effect of this major change in mastitis management. Therefore, this study aimed to provide insight into the level of implementation of SDCT in 2013 in the Netherlands, the methods used for selection of cows for DCT, SDCT in relation to mastitis incidence and antimicrobial usage, and the attitude and motivation of farmers towards SDCT.

Materials and Methods: Data on clinical mastitis, bulk milk somatic cell count (BMSCC) and antimicrobial usage were collected prospectively in 179 farms, and data on subclinical mastitis were available from 177 farms. Farms were classified based on the percentage of cows on the farm that were dried off with antibiotics. Dry cow management and the mindset of 179 Dutch dairy farmers regarding the implementation of SDCT in the year 2013 were evaluated retrospectively using a detailed telephone questionnaire, that contained items about application of DCT, technical knowledge on SDCT, attitude towards DCT, and items on dry period management and farmer characteristics. There were open questions, questions with pre-defined answer categories, as well as statements that the farmers rated on a 5-point Likert scale. The relationship between the

level of SDCT, antimicrobial usage and udder health parameters was analyzed using a Kruskal-Wallis analysis.

Results: During 2013, 44 farmers (25%) applied blanket dry cow therapy (BDCT) and 135 (75%) applied SDCT. Of all participants 113 farmers (64%; group 1) dried off >60%, and 64 farmers (36%; group 2) ≤60% of cows with antibiotics.

There was no significant difference in the incidence rate of clinical mastitis, nor in the prevalence of subclinical mastitis in heifers, between the groups 1 and 2. Group 2, however, had a significant higher BMSCC ($p < 0.004$) and a higher incidence of subclinical mastitis in multiparous cows ($p < 0.04$) than group 1. Between the groups a difference in total antimicrobial usage was found, obviously due to a difference in use of dry cow antibiotics. There was no difference in antimicrobial usage for intramammary treatment of clinical mastitis between both groups.

Conclusions: The mindset of farmers towards SDCT was surprisingly positive, although farmers were concerned about the udder health situation and were uncertain on what to expect. Nevertheless, most of them indicated they understood the reason of the ban on preventive use of antibiotics, and acted proactively towards the decrease of the use of dry cow antibiotics. Based on differences in the udder health situation per farm as well as differences in the attitude of farmers, practical implementation of SDCT on the dairy farm needs clear, scientifically based directives as well as a custom made advice.

References: C.G.M. Scherpenzeel, I.E.M. den Uijl, G. van Schaik, R.G.M. Olde Riekerink, J.M. Keurentjes, and T.J.G.M. Lam. Evaluation of the use of dry cow antibiotics in low somatic cell count cows. *Journal of Dairy Science*, 2014, 97, 3606–3614.

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Staphylococcus aureus genotype B: sanitation of positive dairy herds using a qPCR approach

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Objectives: *Staphylococcus* (*S.*) *aureus* is one of the most important pathogens responsible for intramammary infections (IMI) in cattle worldwide. *S. aureus* genotype B (GTB) is contagious (herd problems) whereas the remaining genotypes infect individual cows and quarters. *S. aureus* GTB causes high economic costs and is a concern for food safety (enterotoxin production). Eradication of this pathogen in Swiss dairy herds would be, therefore, highly beneficial. For this reason, a highly sensitive and specific qPCR assay to detect *S. aureus* GTB in milk was applied for sanitizing GTB-infected herds and was compared to sanitation using classical bacteriology.

Materials and Methods: Using bulk tank milk, we selected 18 herds positive for *S. aureus* GTB. They were randomly allocated to one of 2 sanitation groups: for the “bacteriology group” (9 herds), phenotypic bacteriology was performed on aseptically collected milk samples, while for the “qPCR group” (9 herds) the qPCR approach (Boss et al., 2011; Voelk et al., 2014) was applied for the analysis of clean, non-aseptic milk samples. The milk of all lactating cows was periodically analyzed during the whole sanitation time (9 months) at different time intervals for the two sanitation groups. For the “bacteriology group” the classical sanitation procedure was applied as implemented by Kirchhofer et al. (2011). For the “qPCR group”, the milk of the 4 quarters of each cow was analyzed

monthly. For both sanitation procedures, cows were allocated to 3 groups: group 1 (GTB negative), group 2 (GTB unclear: cows after calving, after a therapy or new on farm) and group 3 (GTB positive). Cows of group 1 were milked first, cows of group 3 last in the milking order. A limited number of on-farm measures concerning milking hygiene and management were implemented on all farms during the whole observation period. Beneficial properties of the qPCR method are: i) it requires only clean milk samples; ii) single milk sampling is adequate for detection of IMI caused by *S. aureus* GTB; iii) high laboratory throughput.

Results: In general, GTB sanitation of herds was possible for both the “qPCR” and the “bacteriology” group. Sanitation by qPCR allowed sampling of large herds (>120 cows) by one person without disturbing the milking process, and the samples of these herds were analyzed within 2 to 3 days. In contrast, sanitation by standard bacteriology was reasonable for herds up to 30 cows as the aseptic sample collection and the laboratory work are very time consuming. After having learnt novel biological phenomena on 2 farms, the rate of new infection was zero for all the remaining herds during the whole sanitation period of the “qPCR group”. All herds could be sanitized if the farmer was willing to cull cows with treatment failure. The same was also true for the “bacteriology group”.

Conclusions: Sanitation of GTB-positive herds can be successfully performed by both analytical methods. However, sanitation by the bacteriological method is restricted to small herds under research conditions. In contrast, sanitation by qPCR is simple, suitable for routine purposes and may include even large herds as sample collection is massively facilitated and as analysis in the laboratory is fast. These beneficial properties of the qPCR method allow for the first time to envisage area-wide sanitation of contagious mastitis caused by *S. aureus*.

Udder Health & Milk Quality

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Factors Associated With Marketed Milk Production Recovery After Treatment Of Naturally Occurring Acute Coliform Mastitis

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Objectives: Milk production loss after recovery from acute coliform mastitis causes major economic losses for dairy industries. Therefore, an effective treatment that minimizes the adverse impact of coliform mastitis on milk yield is urgently required. Declines in milk production and composition are caused by multiple factors, including cow factors, microorganisms, and treatments, but the influence of each factor has not been determined. The aim of this study was to identify possible risk factors for subsequent milk loss after recovery from naturally occurring acute coliform mastitis.

Materials and Methods: Fifty-three Holstein-Friesian cows were enrolled in this study. All were diagnosed with acute coliform mastitis that was classified as moderate at the first examination and cured 14 days later. Survey target data including cow age, days in milk, systemic and local signs, and initial treatment were collected. Initial treatment data included body temperature, systemic nonsteroidal anti-inflammatory

drug (NSAID) administration, systemic and local antibiotic administration, systemic and local steroid administration, and mammary irrigation regimen after frequent milking out. Systemic and local antibiotics were categorized into three groups: none, fluoroquinolones (FQs), and others. Thirty days after clinical cure, owners were interviewed about milk yield recovery and marketing of milk.

The main outcome measure was milk production in a treated quarter (marketed milk or complete loss). The differences between outcome and continuous/categorical variables were examined first. A logistic regression model was used to analyze the strength of the associations. Continuous variables (age, days in milk, and body temperature) in both groups were examined using the Mann-Whitney U test. Categorical variables were assessed by Fisher's exact test and then chosen based on their statistical significance ($P < 0.25$).

The Kaplan-Meier method with the log-rank test was used to compare survival (time to event) curves between outcomes.

Results: In the analyses of continuous variables, no statistically significant differences were noted. In the analyses of categorical variables, three categorical variables (systemic administration of antibiotics, intramammary administration of antibiotics, and mammary irrigation) were selected for multivariate logistic regression analysis. Systemic administration of FQ was the only independent predictor associated with outcome, compared with the group that received no antibiotic (odds ratio, 21.7; 95% confidence interval, 1.48-317.00). There were no significant differences in any other variables. Survival curves were significantly different between outcomes (harvesting of marketed milk or complete loss of milk in a quarter) ($P = 0.046$, time-to-event analysis, log-rank test).

Conclusions: Systemic administration of FQ as the initial treatment of acute coliform mastitis classified as moderate was significantly associated with the prevention of subsequent milk loss. Though statistical association does not guarantee causation, our results can be taken into account in future studies. This knowledge will allow practitioners to provide appropriate treatment, and infected cows may be able to live longer by maintaining productivity.

Udder Health & Milk Quality

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The Effect of Supplementation with Organo-Modified Clinoptilolite on the Colostrum Quantity and Quality in Holstein-Friesian Heifers

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Objectives: Providing adequate amount of high quality colostrum to the newborn calves is an important issue in dairy farming. Colostrum from the first lactating dairy cows on our commercial dairy farms is usually discarded to the general opinion that quantity and quality is insufficient to provide adequate passive immunity transfer. In recent years the use of natural and synthetic zeolites in animal nutrition has increased. The

objective of this study was to determine the effect of supplementation with organo-modified clinoptilolite on the colostrum quantity and quality in Holstein-Friesian heifers.

Materials and Methods: Total number of 19 Holstein-Friesian heifers at the commercial dairy farm were daily supplemented with 150 g of organo-modified clinoptilolite (Minazel plus, Patent.co, Belgrade, Serbia) during the late pregnancy (25±5 days before parturition until 2 days after parturition). Control group of animals were 16 non supplemented Holstein heifers. Total volume of colostrum from 1-4 milkings (12 hours interval, first 48 hours after parturition) was recorded and 200 ml of colostrum samples were collected and stored at -20°C until analysis. The colostrum immunoglobulin G (IgG) concentration was determined using radial immunodiffusion (RID) method (RID kit, The Binding Site group Ltd, Birmingham, UK), and total mass of colostrum IgG was calculated. Statistical analysis was performed using GraphPad Prism statistical package, and after testing of normality the differences between mean values of all data obtained from supplemented and control group were tested using unpaired t-test (or unpaired t-test with Welch's correction, if normality test was negative). Regression exponential curves for colostrum IgG concentrations were determined and tested.

Results: Highest volume of colostrum was obtained at the 4th milking and lowest at 2nd milking in both groups (6.3±1.7L in both groups at 4th milking; 3.4±1.7L and 3.9±1.7L in control and supplemented group at 2nd milking, respectively). There were no significant differences in the total volume of colostrum at 1-4 milking between supplemented and control group of animals. However, colostrum IgG concentration and total mass were significantly higher at the first and second milking in supplemented animals (IgG concentration: 184±40g/L:130±34g/L, 95±54 g/L:57±30 g/L, $p < 0.05$; IgG total mass: 689±393g:436±222g, 301±156g:184±109g, $p < 0.05$; 1st and 2nd milking in the supplemented and control group, respectively). There was a significant difference between regression curves of the mean IgG concentration at 1-4 milking between supplemented and control group ($y = 381.9e^{-0.83x}$, $R^2 = 0.985$; $y = 300.2e^{-0.89x}$, $R^2 = 0.996$, $p < 0.05$, supplemented and control group, respectively).

Conclusions: The results of this study indicate that colostrum quantity (total volume) in Holstein-Friesian heifers is not significantly affected by the supplementation with organo-modified clinoptilolite. However, colostrum quality (assessed by concentration and total mass of colostrum IgG) are significantly increased by supplementing Holstein-Friesian heifers with the organo-modified clinoptilolite during late pregnancy.

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Genotypic diversity and lineage-specific expression of virulence traits in bovine-adapted *Staphylococcus aureus*

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Objectives: The objective of this study was to determine the genotypic diversity of bovine-adapted *Staphylococcus aureus* isolated from cases of clinical mastitis in Ireland and characterise the association between genotype and expression of a range of virulence attributes.

Materials and Methods: A panel of clinical mastitis-associated *S. aureus* isolates ($n = 120$) was tested for their clonal diversity by Multi-Locus Sequence Typing (MLST). Virulence gene content of the isolates was determined by DNA microarray analysis using the Alere Identibac *S. aureus* array. The genome of 8 isolates was sequenced using an Illumina MiSeq with 300 bp paired-end reads. Antimicrobial susceptibility testing for Penicillin (6 µg/10 IU), ampicillin (10 µg), amoxicillin and clavulanic acid (20 µg + 10 µg), oxacillin (1 µg), tetracycline (30 µg), kanamycin (30 µg), neomycin (30 IU), ceftiofur (30 µg), enrofloxacin (5 µg), erythromycin (15 µg), clindamycin (2 µg) and cefalexin (30 µg) was carried out using the disk diffusion method according to CLSI guidelines. The ability of the isolates to form a biofilm was determined by the microtitre plate assay. The ability of the isolates to adhere to and invade bovine mammary epithelial cells in vitro was quantified 3 hours post-infection by flow cytometry while quantitative sampling by ELISA of the levels of IL-1 β , IL-6, IL-8 and TNF α at 12 hours post-infection was used to determine the in vitro host response to infection.

Results: In total, 15 different sequence types (STs) were identified, which grouped into 3 lineages CC97 ($n = 64$), CC151 ($n = 42$) and ST136 ($n = 14$). DNA microarray analysis and whole genome sequencing revealed a genomic rearrangement within isolates from CC97, with a sub-group related to ST71 showing evidence of an IS431 insertion element having replaced approximately 30 kb of DNA including the intercellular adhesin (*ica*) operon and the histidine biosynthesis genes. This resulted in histidine auxotrophy among the ST71-like isolates. As this sub-group differed from typical CC97 in a number of virulence genes, including the *ica* operon, collagen-binding protein (*cna*) and capsule type, they were treated as a separate lineage for analysis purposes resulting in four lineages, CC71 ($n = 42$), CC97 ($n = 22$), CC151 ($n = 42$) and ST136 ($n = 14$). Over half of the isolates (53%) demonstrated resistance to penicillin and ampicillin but all were susceptible to the other antibiotics tested. Penicillin and ampicillin resistance was associated with both CC71 and CC97. There were no differences among the lineages in ability to bind to or invade bovine mammary epithelial cells in vitro although there were significant differences between individual isolates. There was a significant difference between the lineages in ability to form a biofilm ($P < 0.0001$) with ST136 forming the strongest biofilm while CC151 formed the weakest biofilm. CC97 and CC71 also induced a significantly stronger IL-1 β and IL-6 host immune response from bovine mammary epithelial cells in vitro than CC151 or ST136 ($P < 0.01$).

Conclusions: The genotypes of *S. aureus* associated with clinical mastitis in Ireland were restricted to a small number of known bovine-adapted lineages. A genomic rearrangement within one of the lineages was discovered that may be responsible for diversification of this sub-group to an emerging bovine-adapted group. With the exception of penicillin and ampicillin resistance, resistance to other antimicrobials was not detected. The lineages differed in their ability to form a biofilm and their ability to induce a host immune response in vitro indicating the potential for a strain-specific immune response to infection.

Udder Health & Milk Quality

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Evaluation of the effect of clinical mastitis on udder firmness in lactating dairy cows

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Objectives: Detection of infected udder quarters precedes the recently intensively discussed antibiotic therapy of clinical mastitis (CM). Determination of udder firmness is a frequently applied method to diagnose CM. The increasing number of automatic milking systems where detection of CM is carried out automatically requires improved detection methods. We set out to (1) determine if udder firmness determined with a validated dynamometer can be used as a cow-side detection tool for CM and (2) to establish objective firmness thresholds for the differentiation between cows without and with CM.

Materials and Methods: The study was conducted on a commercial dairy farm milking 1,200 Holstein-Friesian dairy cows. Cows were milked 3 times a day. The annual milk yield was 10,140 kg. Cows with signs of CM (i.e., clots or flakes in foremilk samples) were separated into a mastitis pen. During the study period, average somatic cell count was 230,000 per ml bulk tank milk and incidence of CM was 24 cases per 100 cow-years.

Udder firmness of 95 healthy cows and 45 cows with CM in one of their hind quarters was measured by the same investigator before and after milking with a dynamometer (Penefel DFT 14; Agro Technologies, Forges-les-Eaux, France). The dynamometer was used according standard operating procedures. Milk was sampled and cultured using standard microbiologic methods.

The time (before or after milking) and location within an udder quarter (upper, middle, lower measuring point) for diagnosing CM was tested. Further calculations were performed using the difference in udder firmness between both hind quarters of a given cow (Δ firmness) as the primary outcome. Influencing factors on Δ firmness were tested by generating a generalized mixed model with CM, milk yield per day, DIM, parity and gram staining characteristics of sampled bacteria as included factors. A receiver operating characteristic (ROC) curve was calculated to evaluate the performance of Δ firmness as a diagnostic tool for CM and provide thresholds for firmness values to differentiate between cows with and without CM.

Results: Median (and interquartile range (IQR)) udder firmness in quarters without (1.867 kg, 1.243 to 2.671 kg, $n = 6,340$) and with CM (1.948 kg, 1.213 kg to 2.819 kg, $n = 1,220$) was similar before milking ($P = 0.675$). After milking, udder firmness differed ($P < 0.001$) between quarters without (0.636 kg, 0.514 to 0.783 kg) and with CM (1.036 kg, 0.704 to 1.702 kg).

Firmness of both hind quarters within a cow without CM did not differ ($P = 0.369$) whereas firmness of both hind quarters within a cow with CM differed ($P < 0.001$).

Median (and IQR) firmness of the middle measuring point was 0.722 kg (0.600 to 0.864 kg) in quarters without CM and 1.297 kg (0.844 to 1.959 kg) in quarters with CM ($P < 0.001$). This middle measuring point was the firmest point in quarters without CM. In quarters with CM, there was no difference in firmness of the middle and the lower measuring point (1.203 kg, 0.771 to 2.053 kg, $P = 0.257$).

Median (and IQR) Δ firmness differed ($P < 0.001$) between cows without (0.098 kg, 0.030 to 0.216 kg) and with CM (0.756 kg, 0.170 to 1.914 kg). In cows in their 2nd and higher lactation, Δ firmness after milking was affected by CM ($P < 0.001$), milk yield per day ($P = 0.001$) and DIM ($P = 0.001$). Except for CM ($P < 0.001$), other tested factors did not affect Δ firmness after milking in cows in their 1st lactation. A threshold (area under the ROC curve) of 0.282 (0.722) and 0.425 kg (0.817) Δ firmness provided the best combination of sensitivity and specificity i.e., identified 64% of 1st lactating cows and 62% of older cows as truly suffering from CM, respectively.

Conclusions: The increased firmness of an udder quarter with CM does not affect firmness of its neighboring quarter. To diagnose CM, udders should be examined after milking using a defined measuring point located in the middle or lower part of a given quarter. Furthermore, different firmness thresholds for 1st lactating and older cows should be

used to reduce bias by parity. In conclusion, assessing udder firmness with the dynamometer can be a promising tool in daily udder health management.

Udder Health & Milk Quality

P01-001-206

Prevalence and Risk Factors for Udder Cleft Dermatitis in Swedish Dairy Cows

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Objectives: Udder cleft dermatitis (UCD) occur in the anterior parts of the udder of dairy cows. Cows with UCD may have increased risk of clinical mastitis, and associations between UCD and digital dermatitis (DD) have also been suggested. However, there are few large-scale studies investigating prevalence, etiology or risk factors. As the lesions are likely to have negative effects on animal welfare preventive measures are warranted. The objectives of the study were to investigate the prevalence of UCD in Swedish dairy herds, identify herd- and cow-level risk factors associated with UCD, and analyze associations between UCD and mastitis, DD and culling.

Materials and Methods: A random sample of 100 free-stall dairy herds with 50-210 cows, milking parlor and affiliation with the Swedish official milk recording scheme were included in the study. Each herd was visited once. Every second to third milking cow was examined and registered as having no UCD (no visual or palpable skin damages), mild UCD (small papules/pustulae, hyperkeratosis, small crusts and/or serum transudation) or severe UCD (large palpable skin damages, large crusts, pus, blood, deep skin wounds and/or proliferations). The cows were also examined for udder traits and the hygiene of the hind limb and udder was scored. Information on individual cows, herd management and cow environment factors was obtained on farm (via registrations and a questionnaire), and via the official milk recording scheme. Cow level risk factors included age, breed, days in milk, milk yield, udder conformation, hygiene, milk somatic cell count (SCC) and urea, hock lesions, claw disorders and reported diseases. Associations with mastitis cases 30 days before and after the visit, and culling up to three months after the visit were also analyzed. Herd level risk factors investigated were herd size, production system (organic or non-organic), average milk production, breed, bulk milk SCC and urea, stocking density, cubicle size, bedding material, routines for cleaning, milking (including use of water at milking), claw trimming and feeding, length of pasture period, problems with ectoparasites and registered veterinary treated diseases (with special regard to mastitis and DD).

Results: The total cow prevalence of UCD was 29% (1109 of 3775 cows). The majority (69%) of the UCD cases were mild while 31% of the cases were severe. The average herd prevalence of all UCD cases was 31% (range 0-64%), while the average herd prevalence of severe UCD was 10% (range 0-38%). The within-herd proportion of mild and severe cases varied markedly. Some herds had a high proportion of mild UCD with no or few severe cases. Other herds had similar proportions of mild and severe UCD, while a few herds mainly had severe cases. Only one herd had no cases of UCD. Associations between UCD and the risk factors are analyzed using univariable and

multivariable regression models and these results will be presented at the congress.

Conclusions: The fact that 10% of the cows suffered from severe UCD emphasizes the need for preventive measures. The UCD prevalence varied markedly between herds, as did the proportions of mild and severe UCD within herds, indicating that management or other herd factors may be important for the occurrence of UCD. Such factors, if identified, could be used to design preventive measures to reduce UCD prevalence. Longitudinal studies and microbial investigations of UCD lesions are needed to increase the understanding of why and how the lesions develop, persist and heal, and will also be performed within the project.

Udder Health & Milk Quality

P01-001-207

Understanding communication on mastitis management: could Motivational Interviewing aid in the uptake of advice?

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Objectives: Mastitis is one of the most significant causes of morbidity and mortality of the adult dairy cow (Ruegg 2011). Compliance with veterinary recommendations is critical to tackle incidence rates on farm (Green et al. 2007), yet improving uptake of advice in daily practice is a challenge (Jansen et al. 2010). Similar challenges are faced by the medical profession, who are increasingly employing an evidence-based communication methodology called Motivational Interviewing (MI) to improve public health. This research examines vet-farmer communication on disease, with the objective of establishing whether MI communication skills are currently in use.

Materials and Methods: Role play sessions reflecting consultations on lameness and mastitis were recorded between cattle veterinarians (n=15) recruited from two UK practices located in South West England and an actress experienced in role play in medical and veterinary education. The actress was provided with a character and farm profile reflecting a 'typical' UK situation, and veterinarians were provided with a short excerpt on the disease issue/risk factors on the farm and evidence to encourage them to broach a broad topic area of change with the farmer. Consultations lasted an average of 11.2 minutes (range 7.7 to 14.9).

Consultations were analysed using the MITI 4.2.1, a treatment integrity measure for clinical trials of MI. In this system, verbal interactions are firstly coded for frequencies of verbal behaviours: Giving Information, Persuading, Persuading with Permission, Questions, Reflections (simple/complex), Affirmations, Seeking Collaboration, Emphasising Autonomy and Confronting. Secondly, global scores are assigned on a five point Likert scale (from 1: low proficiency to 5: high proficiency) to characterise the entire consultation in relation to MI foci: Cultivating Change Talk, Softening Sustain Talk, Partnership and Empathy. To meet the level of 'basic competency' in MI, veterinarians required a mean score of 3.5 in Relational globals (Partnership, Empathy) and 3 in Technical globals (Cultivating Change Talk, Softening Sustain Talk), a Reflection to Question ratio of 1:1 and a 40% Complex Reflection percentage (of total Reflections). Coding was performed directly from audiotapes.

Results: MI communication skills were recorded within these veterinary consultations, yet no veterinarian was classified at a level of overall 'basic competency'.

Verbal behaviours

Veterinarians predominantly relied on Persuasion, Questions and Giving Information in their consultation approaches. Communication behaviours inadherent with MI (Persuasion and Confrontation, n=126) far exceed total MI adherent behaviours (Affirmations, Seeking Collaboration and Emphasising Autonomy, n=15), whilst no veterinarian achieved 'basic competency' with regards to Question: Reflection ratio (goal: 1:2). However, Reflections were found in nine of the fifteen veterinarian-farmer interactions.

Consultations approach: global scores

One veterinarian of the fifteen met the criteria for basic competence in MI with a mean score of 3.5 in Relational globals (Partnership, Empathy) and a mean score of 3 in Technical globals (Cultivating Change Talk, Softening Sustain Talk). However, mean global scores overall were low - Relational score = 1.6 (range 1-3.5) and Technical score = 1.7 (range 1-3).

Conclusions: These data suggest that current veterinary communication practices do not employ MI methods overall. However, some MI skill naturally occurs in practicing cattle veterinarians, indicating the feasibility of this methodology's utilisation within this context. Further training in this methodology could enhance the advisory process for both veterinarians and farmers, thereby improving the uptake of advice and reducing the incidence of mastitis, amongst other management challenges, on UK dairy farms.

Udder Health & Milk Quality

P01-001-208

Pathogens Agents of the Udder Isolated Milk Reception Boat of Goats with Mastitis the Municipality of Tanhuato Michoacan

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Objectives: Identify the pathogens agents of the udder isolated milk reception boat of goats with mastitis the municipality of Tanhuato, Michoacán.

Identify the presence of *Staphylococcus aureus* as the main causative agent of mastitis in goats the municipality of Tanhuato, Michoacán.

Materials and Methods: The number of sample units (n = 49 stables) was calculated using a random model with proportional distribution function of the system characterization by small-scale dairy and major conglomerates. Sampling within herds was per cluster, ie a milk sample pot of receipt of each production unit was taken. the sampling of milk using for this test tubes sterilized sealing plug in which the necessary identification previously stable are scored. The test tube is placed inclined to keep out dirt. The sampling was made directly from the milk storage tanks. The sample tube was filled with two thirds at most. Finally, the sample tubes were placed on a rack placed in a sealed cooler for

further transport to the laboratory of bacteriology of USAD of the Faculty of Veterinary Medicine and Animal Husbandry for immediate processing or refrigeration at 4 ° C. The processing of the milk samples obtained was held on sampling or the next day. Samples were seeded in 110 staphylococcal agar, azide blood agar and Mc Conkey agar. Then, the agar plates were incubated at 37 ° C and examined after 24 and 48 hours. The isolates were identified by colonial morphology, Gram stain, catalase test, coagulase test and the test of mannitol and gelatin. A isolates Mc Conkey agar, they were made corresponding biochemical tests for identification. The tests used were: Urea, citrate, SIM (Sulfide Indole Motility) test, Agar triple-sugariron for its acronym in English TSI, Middle MRPV (methyl red Voges-Proskauer).

Results: 49 samples of the boat receiving equal number of dairy herds goats, of which a total of 61 bacterial strains (100%) were isolated were collected. Of these, 29 (47.54%) were Gram-positive pathogens, while 32 (52.46%) Gram-negative pathogens. On the results of bacteriological analysis of the milk samples obtained in this study (Table 1), the staphylococci were identified as the main etiologic agents Gram-positive mastitis, among which the *S. aureus* as the main causal agent 21 insulation, equivalent to 34.43%, as well as coagulase negatives which mainly found pathogen was *Staphylococcus epidermidis* with 8 isolates representing 13.11%, results appear to be similar to an investigation by Ferrer, et al (1993) who obtained 12 cases, equivalent to 11.5% of *Staphylococcus aureus* and 46 equivalent to 44% divided into agents coagulase negative cases.

Conclusions: It is concluded that *Staphylococcus aureus* was the main pathogen found in samples of milk pot receiving goats Tanhuato municipality, Michoacan, followed by *Proteus mirabilis*, coagulase Negative (*Staphylococcus epidermidis*) and agents Gram negative pathogens: *Citrobacter intermedium*, *Proteus rettgeri*, *liquefaciens serratia*, *Alcaligenes faecalis* and *Citrobacter freundii*.

Udder Health & Milk Quality

P01-001-209

An investigation of the incidence of milk leakage after dry-off in Commercial Dairy Herds around Europe

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Objectives: The objective of the study was to investigate the incidence of cows leaking milk during the first two days post dry-off on commercial dairy farms around Europe and to investigate the relationship between milk leakage and milk production.

Materials and Methods: This study was carried out in commercial dairy farms in France, Germany, Italy, The Netherlands, Belgium, Spain, Czech Republic and Denmark. A total of 1,142 cows from 41 farms were included in the study. All cows had a satisfactory general health based on a physical examination including clinical mastitis, four functional quarters and were not treated with internal or external teat seal. Milk leakage was observed at three visits (V): between 20 to 24 hours (V1), 30 to 34 hours (V2) and 48 to 52 hours (V3) after the dry-off. Cows had

to stand in headlocks and were observed twice per visit for 30 seconds each. Milk leakage was considered to be present when a stream of milk was coming from a teat, when a milk drop on the teat was present or when milk was present on the ground under the udder. Milk production data during the last 24 hours before dry-off was obtained from the records on the milking system. The individual cow was the experimental unit, whereas the quarter was the data collection unit. Generalized linear models with random effects for farm were used to test the relation between milk leakage and production.

Results: Milk leakage within two days after dry-off was observed in 279 out of the 1142 cows, resulting in a mean milk leakage incidence per farm of 24.4% (95% CI: 21.9% - 26.9%). Cows with observed milk leakage, had on average 1.6 leaking teats (95% CI: 1.56 - 1.66). In 55.3% of the cows leaking milk one quarter was leaking, in 32.3% two quarters, in 8.3% three quarters and in 4.1% four quarters. Rear quarters turned out to have 4.3 times as many odds on milk leakage than front quarters (95% CI: 3.3 - 5.7). Most milk leakage (on average 30,1% of the cows per farm) was observed between 30 and 34 hours after dry-off compared to on average 14,7% of the cows per farm between 20 to 24 hours and 17,2% between 48 to 52 hours, respectively. The last recorded yield 24h before dry-off was significantly associated with milk leaking. Cows with a higher last recorded yield had 2.6 (95% CI: 1.61 - 4.27) higher odds on milk leakage than cows with a lower last recorded yield, even though the association was not completely linear.

Conclusions: Results show that the incidence of milk leakage in these European dairy farms is 24.4% average. Due to the link between the cow milk production the last day before dry-off and the incidence of milk leakage after dry-off, the data indicate that higher milk production at dry-off is a predictor of milk leaking after dry off.

Udder Health & Milk Quality

P01-001-210

Effect of a single injection of Cabergoline (Velactis®) on the incidence of milk leakage after dry-off in large dairy herds in Mexico

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Objectives: The objective of the study was to investigate the efficacy of one injection of Cabergoline (Velactis®, Ceva Sante Animale) at the moment of dry-off on the percentage of cows leaking milk during the first two days after the dry-off in large dairy herds in Mexico. The relationship between milk leakage and milk production was also investigated.

Materials and Methods: This multi-site, randomized and blinded study was carried out in two large commercial dairy cows in Mexico. A total of 1,072 cows from 2 farms were enrolled in the study. The involved cows for the study had a satisfactory general health based on a physical examination including clinical mastitis, four functional quarters and were not treated with internal or external teat seal. The cows were randomized to receive 2 treatments to evaluate the incidence of milk leakage. After the last milking 535 cows received a single i.m. injection of 5 mL of a solution containing 5.6 mg of cabergoline (Velactis®-VEL-) and 537 received an equivalent volume of placebo (only excipient -PLA).Milk leakage was observed at three visits: between 16 to 20 hours, 24 to 28 hours and 42 to 46 hours after the dry-off. For this, cows had to stand in headlocks and were observed twice per visit for 30 seconds each.

Milk leakage was considered to be present when a stream of milk was coming from a teat, when a milk drop on the teat was present or when milk was present on the ground under the udder. Milk production data during the last 24 hours before dry-off was obtained from the records. The individual cow was the experimental unit, whereas the quarter was the data collection unit. The statistical unit was the cow. Student t-Test was used to compare milk production between groups and Chi - test was used for binomial comparisons between groups.

Results: Average milk production at dry-off did not differ (p=0.79) between treatment groups, and it was 17.7 ± 10.6 and 17.5 ± 10.3 for cows on PLA or VEL respectively. After the dry-off, milk leakage was observed in 29.2% (95% CI: 25.4% - 33.2%) PLA (157 out of 537) and 10.8% (95% CI: 8.3% - 13.8%) VEL (58 out of 535) respectively (p

Conclusions: Data show that the treatment with a single i.m. Injection of 5 mL of a solution containing 5.6 mg of cabergoline (VEL; Velactis®, Ceva Sante Animale) results in a relative risk reduction of 63% in milk leakage on the two first days after dry-off compared with placebo group.

Udder Health & Milk Quality

P01-001-211

An investigation to determine visual presence of milk leakage in US commercial dairy farms after dry-off

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Objectives: The objective of this study was to determine the visual presence of milk leakage on commercial U.S. farms at 4 hours, 8 hours, 12 hours, 24 hours, 36 hours and 48 hours after the last milking at drying off in dairy cows.

Materials and Methods: This was a multi-site survey to determine the presence of milk leakage after the last milking at dry-off. The animal phase of the study was conducted at three commercial dairies in California, Michigan and New York. A total of 312 Holstein cows were included in the study. Cows were observed in weekly cohorts according to the dairy's current dry-off schedule. Each site enrolled approximately 100 animals (Site A- 100; Site B- 108; Site C- 104) where each cohort included no more than 35% first calf heifers. All cows had a satisfactory general health based on a physical examination including evaluation for clinical mastitis, and had not been treated with internal or external teat seal. Cows with more than one non-functioning quarter were excluded from the study. Cows were observed and milk leakage was assessed after the last milking at dry off at the following time points: 4 hours, 8 hours, 12 hours, 24 hours, 36 hours, and 48 hours. The milk leakage visual observation was made without any manipulation of the teats. A cow was positive for milk leakage if dripping or squirting of milk was observed from one or more teats directly within the lock-up, stanchion or freestall. Milk leakage was observed and evaluated statistically on a cow basis.

Results: Milk leakage within 2 days after dry off was observed in 81 out of 312 cows, resulting in a mean milk leakage incidence per farm of 26%. The site-specific incidence varied from 19% to 35% during the full observation period of 48 hours. The observation of leakage was related to observation interval, with a higher incidence rate of leakage at the later 24 hour (13.7%), 36 hour (21.1%) and 48 hour (14%) observation intervals.

Conclusions: Results show that the incidence of milk leakage in these USA commercial dairy farms is 26% average. Researchers have been identified milk leakage as a major risk factor for the acquisition of new intramammary infections during the dry off period. Strategies to reduce milk leakage should be considered to improve udder health.

Udder Health & Milk Quality

P01-001-212

Survivability of *Mycoplasma* spp. Isolated from milk samples from South Australia in different storage conditions

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Objectives: The study aims to evaluate the recovery of *Mycoplasma* spp. isolated from milk samples under different storage conditions. This study should provide veterinarians and farmers in designing appropriate procedures to keep milk samples in order to use them for *Mycoplasma* detection. Furthermore, the study will compare between some cryoprotectants to avoid the intra and extracellular freezing injuries of *Mycoplasma* spp.

Materials and Methods: Four different regimens of storage conditions were used to compare the recoverability of *Mycoplasma* spp isolates after various periods of time. Samples were collected from a single dairy farm in Mount Gambier, South Australia. According to the culture and PCR tests, thirty of *Mycoplasma* positive milk samples have been selected for storage-recovery test. Each of these samples was aliquoted into twelve (0.5 mL) Eppendorf tubes for each experiment. The first experiment was carried out using 40% of glycerol, 10% Dimethyl Sulphoxide (DMSO) and 50% (v-v) of 10 days *Mycoplasma*-culture broth. Similarly, Foetal Bovine Serum (FBS) was used in the second experiment instead of glycerol with 40% concentration in addition to 10% DMSO and 50% of the *Mycoplasma*-positive culture. Samples from the first and second experiments were stored in -80°C freezer. Freezing of the milk samples at -20°C was used as the third method, while the last storage method was using a 4°C fridge. After thawing at ambient temperature, 250 µL of each aliquot were cultured after 1, 2, 4 and 8 weeks interval to record the growth on agar plate (Oxoid Ltd.). Colony forming unit (CFU) was calculated for each sample regimens and compared with the initial culture results using kappa agreement procedure in SAS.

Results: Results have shown a variety of *Mycoplasma* recoverability using the four different storage techniques for different times. *Mycoplasma* survived well in Glycerol and DMSO with 100% recovery rate for the duration of the experimental period. The combination of FBS and DMSO has shown lesser survival with 90%, 70%, 60% and 30% for week 1, 2, 4 and 8 respectively at -80°C. The recovery rate declined under -20°C storage with 80%, 75%, 66% and 30% for week 1, 2, 4 and 8 respectively. The lowest survivability was observed with the fridge milk samples with 50%, 30%, 10% and 0%, respectively.

PARTS OF THE RESULTS ARE STILL EXPECTED. THEY WILL BE AVIALBLE BY THE END OF FEBRUARY 2016. THEREFORE, SOME OF THE VALUES ABOVE MAY CHANGE.

Conclusions: Unless using fresh milk samples, a combination of Glycerol and DMSO has been found as appropriate cryoprotectants for *Mycoplasma* storage. Results of this study may contribute to overcome

the survival challenges of *Mycoplasmas* due to their fastidiousness. Additionally, this methodology can be used for long distance transport of microbiological milk samples. For farmers and veterinarians, storage of milk samples in -20°C appears to be more efficient than fridge.

Udder Health & Milk Quality

P01-001-213

Bacteriological etiology of mastitis in Finland in different housing and milking systems

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Objectives: Knowledge on the bacteriological etiology of mastitis is important for efficient mastitis control and treatment. In Finland, milk samples for bacteriology are taken in most cases of mastitis. Since the year 2010, samples have been analyzed using a quantitative PCR test (PathoProof™ Mastitis PCR Assay, Thermo Fisher Scientific, Finland). The test targets DNA of 15 bacterial species or groups of species. The aim of this study was to define the distribution of pathogens identified by the PCR test in different housing and milking systems.

Materials and Methods: The data consisted of 243,520 aseptic quarter milk samples sent to Valio Laboratory during 2010-2012. The samples originated mainly from subclinical or mild clinical mastitis and were mostly taken by herd staff. The milk samples were taken from 93,530 individual dairy cows on 4,725 dairy farms. Milk samples with DNA of one target only were included in the study. The data containing records of analyzed milk samples were merged with the data from the Finnish dairy herd recording system. In addition to production data, this system includes information on herd and cow factors like housing and milking type.

Results: Both housing and milking system were associated with proportions of different bacteria in milk samples. Coagulase-negative staphylococci (CNS) were the most frequent bacteria found in milk samples. The proportion of CNS in milk samples was bigger in tie stall (45%) compared to cold (38%) or warm (42%) free stall housing, and in automatic milking compared to parlour milking. The proportion of *Staphylococcus* (*S.*) *aureus* in milk samples was bigger in tie stall (24%) and warm free stall (19%) housing compared to cold free stall (14%) housing, and in parlour milking (19%) compared to automatic milking (16%). The proportion on *Streptococcus* (*Str.*) *uberis* in milk samples was bigger in cold free stall (12%) housing compared to warm free stall (9%) or tie stall (9%) housing. On the contrary, the proportion of *Str. dysgalactiae* in milk samples was bigger in warm free stall (9%) compared to cold free stall (6%) and tie stall (7%) housing. The proportion of *Corynebacterium* (*C.*) *bovis* was bigger in cold free-stall (14%) compared to warm free-stall (7%) and tie stall (6%) housing. The proportion of *Escherichia* (*E.*) *coli* in milk samples was bigger in free-stall (cold 7%, warm 6%) than in tie stall (3%) housing. *Str. agalactiae* was an infrequent finding which was associated more commonly with automatic milking (1.5%) than with parlour (0.3%) or tie stall (0.1%) milking.

Conclusions: Both housing (tie stall, cold and warm free stall) and milking system (pipe line in tie stall, parlour and automatic milking) were found to be associated with the proportions of different mastitis causing bacteria in milk samples sent for bacteriological analysis. Housing and milking systems are not independent as certain type of milking

systems are used in certain types of housing systems. Thus, the real causes behind these associations may be the housing and management conditions in barns of different age and size.

Udder Health & Milk Quality

P01-001-214

Comparison of different methods to assess the quality of bovine colostrum

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Objectives: It is vital for new-born calves to obtain good quality colostrum. The main method to assess the quality of colostrum is determining the concentration of immunoglobulin G. Several methods are available to assess this concentration: either in the laboratory, using a colostrometer, or using digital or optical refractometers. The present study had the objective to compare different methods for assessing the quality of bovine colostrum, including a newly developed method of using a standardised funnel.

Materials and Methods: Two different refractometers (optical and digital), one colostrometer and a funnel, as used in the varnish industry were used to assess the colostrum of 124 cows. In addition the lactation of the cow, the time between calving and milking (in hours) and the time between milking and measuring (in minutes) were recorded. After the milking the quality of the colostrum was assessed using an optical and a digital refractometer. Thereafter, the colostrum was frozen and later on thawed again for the examination using the refractometers again, using a colostrometer, using the funnel, determining the density and the IgG-concentration.

Results: Using the concentration of IgG as a gold-standard the different methods reached correlations between 0.42 up to 0.64. Correlations between the refractometers and the colostrometer were higher (up to $r=0.86$). Factors influencing the IgG-concentration were the time period between calving and milking and the lactation of the cow. Freezing and thawing of the colostrum did not have any effect on the measurements.

Although the correlations between the time measured in the funnel and the IgG-concentration was the lowest ($r = 0.42$), the method showed a good discrimination between two classes of colostrum (less than or greater than 50 mg/ml) with an area under the curve in a ROC-analysis of 0.864. Parameters like lactation period and time between calving and first milking were used to evaluate their effect on the times measured in the funnel.

Conclusions: In conclusion, there are several methods available for assessing the quality of bovine colostrum. A newly developed method of using a standardised funnel provides an easy to use technique to validly assess the quality of cows' colostrum.

Udder Health & Milk Quality

P01-001-216

Clinical and economic comparison of immediate blanket treatment versus pathogen based treatment for clinical mastitis in a New York dairy herd

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Objectives: Current practice on many dairies is the immediate treatment of all clinical mastitis (CM) cases with intramammary (IMM) antibiotics or "blanket therapy." Pathogen based mastitis therapy has the potential for targeted use of IMM antibiotics, avoiding their overuse, reducing treatment costs, and increasing saleable milk. The objective of this study was to compare the clinical and economic impacts of blanket IMM therapy with a pathogen based approach. Survival and production indices were used and a partial budget analysis was performed to compare differences in the two approaches using measured outcomes from an on-farm clinical trial.

Materials and Methods: Clinical mastitis (CM) cases at a 3500 cow dairy in New York State Dec 2014-Apr 2015 were included. Cows with systemic signs, prior antimicrobial treatment (<15d), or impending sale were excluded. Cows with CM were randomly assigned to the blanket (BT) or pathogen based (PB) therapy group. Mastitic milk samples were collected daily. Pathogen results were available after 24h by direct upload onto farm computers. Cultures were performed according to NMC guidelines at Quality Milk Production Services. Cows in the BT group received 1 tube of ceftiofur hydrochloride into the affected quarter for 5d. Cows in the PB group received no treatment for the first 24h. Upon upload of results, the following protocol was automatically assigned via Dairy Comp 305: Staph spp., Strep spp., or Enterococcus positive cows were administered 1 IMM tube of cephapirin sodium every 12h for two treatments. Cows positive for other organisms or no growth received no treatment. Cows in either group positive for Prototheca, Mycoplasma, S. aureus, or Strep ag. were culled. Outcomes included days to return to normal milk, days out of the tank, linear score and milk production 8-43d post treatment, removal from the herd <30d or <60d post CM. Analysis was performed using multivariable models in SAS® 9.4. Costs were as follows: \$3.80/tube of ceftiofur, \$3.10/tube of cephapirin, labor=\$15/hr, \$6.00/culture. Milk discard value was based on 27kg/d production for mastitis cows and a \$20/100 pounds of milk price. Herd level costs were based on 5% monthly incidence of CM per 100 lactating cows.

Results: A total of 725 cases of CM were included; 114 cows were excluded due to systemic illness (16%). Distribution of treatments for the 489 included CM events was equal between groups. The proportion of CM cases assigned to the BT and PB that received IMM therapy was 100% and 32.1%, respectively. No statistically significant differences existed between BT and PB therapy in days to clinical cure (means: PB=4.5, BT=4.8). The difference in post-CM milk production between groups was not significant (means: PB = 34.7 kg, BT = 35.3 kg). No significant differences were observed in test day linear scores (LS) between groups (least squares means (LSM) of LS = 4.3 for PB cows and 4.2 for BT cows. Risk of survival 30 d post-enrollment was the same for both groups (Odds Ratio of PB as reference=1.57, 95% CI: 0.66-3.74) as was risk of survival to 60 d (OR=1.39, 95% CI: 0.75-2.57). However, PB cows experienced an average of 3 d fewer out of the tank. Costs associated with clinical mastitis treatment group were as follows:

Direct Cost per Treatment Protocol: BT \$132.05 / PB IMM therapy \$99.95, no IMM therapy \$67.25;

Total Direct Cost by Treatment Group: BT \$132.05 / PB \$78.24 (difference of \$53.81 per case)

Cost of Mastitis Treatment / 100 Cows: BT \$7,923 / CBT \$4,694; Savings per 100 Cows: \$3,229

Conclusions: Almost 70% of moderate and mild CM cases would not have been treated if all CM trial cows were enrolled in a pathogen based protocol. This strategic method of treatment decreased average days out of the tank by 3d for those cows on the pathogen based protocol, with no difference in days to clinical cure, milk yields, and LS post-mastitis event; nor additional risk of culling in the days following. The use of pathogen based therapy to guide the treatment of clinical mastitis reduced treatment costs, increased the volume of saleable milk, and increased cash flow over \$3,000 per 100 cow milking cows with no negative impact on clinical outcomes.

Udder Health & Milk Quality

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Mycoplasma bovis infection and shedding in naturally infected dairy cows

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Objectives: Mycoplasma bovis mastitis has been considered contagious in nature and is transmitted from infected to uninfected udders mostly at milking time. Still, the transmission may less commonly occurs from other source than mammary gland, like the mucosal of nasal cavities, eye and vagina. For these reasons an evaluation of the shedding pathways of M. bovis in infected cows could be useful to better understand the transmission of this microorganism in dairy herd. Thus the aim of this study was to describe the shedding of M. bovis in a group of naturally infected dairy cows.

Materials and Methods: The study was realized in a dairy farm located in the north-east of Italy: the herd size was 240 cows, milked in a herringbone parlour and housed in freestall barns. Due to the finding of M. bovis infection in 2 cows with subclinical mastitis, the milk of each cow was tested for the detection of the pathogen, so others 15 infected cows were found. The farmer decided to cull immediately only 3 infected cows and to postpone the culling of the others 14 cows. These 14 naturally infected cows were sampled monthly for 5 months: milk from each single quarter and nasal and vaginal swabs were collected. A single nasal swab was taken from each nostril, and another swab was taken from vaginal mucosa. Immediately after sampling the swabs were dipped in 2 mL of Mycoplasma Experience (ME) broth medium (ME LTD, Reigate, UK). Milk was collected aseptically in 10 ml tubes and submitted to the following test: standard culture (according to the National Mastitis Council procedure), somatic cells count (SCC) by mean of DeLaval cell counter (DeLaval A.B., Tumba, Sweden) and M. bovis culture. For M. bovis isolation the samples were inoculated onto ME broth medium (ME LTD, Reigate, UK) and incubated at 37±1 °C under 5% CO₂. Broths were checked daily for the presence of turbidity/changing colour, then they were plated on ME agar medium (ME LTD, Reigate, UK), and

checked for the presence of M. bovis colonies. In order to confirm the identification of M. bovis, DNA was extracted from suspect samples and a 16S-rDNA PCR and Denaturing Gradient Gel Electrophoresis (DGGE) method were performed.

Results: At the 1st control 31 quarters (55%) were found infected with M. bovis and 10 cows (71%) had 2 or more infected quarters. Among infected quarters, 13 (23%) had a SCC > 200.000 cells/mL. At the 2nd control 12 cows were sampled (2 were dried-off). Among these, 25 quarters (52%) were found positive and 17 (35%) had a SCC > 200.000 cells/mL. Only 10 cows were positive to M. bovis and 8 cows (67%) had 2 or more infected quarters. Of the 2 negative cows 1 had at the previous control only 1 infected quarter, and the other 2. At the 3rd control were tested 10 cows (1 was dried-off and 1 culled), and 8 were still infected. The numbers of quarters found positive to M. bovis was 19 (48%), and 12 (30%) had a SCC > 200.000 cells/mL. Among the 8 infected cows, 7 had 2 or more infected quarters. At the 4th control only 8 cows were sampled due the culling of the others: among them 3 cows tested negative: 1 was negative also at the 2nd and 3rd test, 1 was at the first control after the dry-off and 1 had tested positive to the previous tests in only 1 quarter. The number of infected quarters was 9 (28%), 7 (22%) had a SCC > 200.000 cells/mL, and 4 cows (50%) had 2 or more infected quarters. At the 5th control among 8 cows 4 were still infected. The number of infected quarters was 5 (16%), and 4 (13%) had a SCC > 200.000 cells/mL. All the vaginal and nose swabs collected were negative for the presence of M. bovis. During the study, the presence of clinical symptoms were observed only in 4 quarters: agalactia in 3 quarters and the presence of flaky sediments in the milk in 1 quarter.

Conclusions: The majority of the cows involved in this study were affected only by a M. bovis subclinical mastitis. The SCC of infected quarters fluctuated during the time of the study also below 200.000 cells/ml, and some quarters recovered from the infection. These findings suggest that M. bovis infection can induce in the mammary gland a self-limiting disease that could be more difficult to recognize, compared to the classical form of mastitis. The M. bovis shedding in these cows occurred only in the milk, demonstrating that only the mammary gland has been colonized by the microorganism.

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Udder Health & Milk Quality

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Comparison of bacteriological finding in milk samples analyzed by 3 different laboratories

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Objectives: The routine diagnostic of mastitis pathogens and antimicrobial resistances mostly includes the bacteriological examination. This gold standard, however, has several limitations. Sterile milk samples are necessary to achieve reliable results and transport between farm and laboratory, often conducted without cooling, might bias the results. An inter-laboratory comparison of Finnish milk laboratories has been published in 2005, however, only spiked milk samples were used in this study. Therefore, the objective of our study was to compare bacteriological results of milk samples collected in the field and shipped to 3 different laboratories.

Materials and Methods: A total of 792 milk samples (175 clinical and 250 subclinical mastitis, 346 healthy) were collected from July 2013 to June 2014 on a commercial dairy farm in Germany. Sterile milk samples were collected according to the guidelines from the International Dairy Federation. Samples were immediately cooled and transported to the laboratory of the Clinic for Animal Reproduction. Upon arrival all samples were vortexed for 15 sec, divided into 3 aliquots and filled into tubes coated with boric acid under sterile conditions. Each tube was sent to 1 of 3 different accredited milk laboratories (Lab A, B, C) that offer routine milk diagnostic for commercial dairy farms and veterinarians. Samples were sent by regular mail without cooling in order to provide influences similar to those samples are exposed to under normal circumstances. All samples arrived within 2d.

Samples in all labs were inoculated on agar plates and incubated. Isolated bacteria were identified based on colony and cell morphology, gram staining, hemolytic pattern, catalase activity and growth on differential and selective media. All samples were examined for the presence of bacteria, yeast and prototheca species and milk somatic cell count was evaluated. Furthermore, antimicrobial susceptibilities for 20 antimicrobials used in mastitis therapy were tested in 102 out of 792 samples.

Association and agreement between labs was estimated utilizing Chi-Square test or Fisher's Exact test, Cohens Kappa test, Spearman's (i.e., antimicrobial susceptibility) and Pearson's (i.e., SCC) Correlation Coefficient and paired t-test.

Results: In 42.3% of all samples the same bacteriological result (i.e., same pathogen or "no growth") was found by all three labs. The same pathogen genus (e.g., Streptococci) was diagnosed in 45.1% of the samples. Comparing only 2 out of 3 labs, Lab A and B identified the same pathogen in 57.4% of all samples (Kappa = 0.335; $P < 0.001$), the differentiation between gram positive and negative was identical in 95.4% of the results. Lab B and C had an agreement of 50.0% (Kappa = 0.308; $P < 0.001$) and 88.7% considering pathogen identification and gram stain, respectively. Best results were achieved between Lab A and C with 72.2% agreement between pathogen identification (Kappa = 0.561; $P < 0.001$) and 100% for gram stain. 17.8% of the results were gram negative. Agreement considering the decision "pathogen" or "no growth" was 62.6%, 63.6% and 74.4% between Lab A and B, B and C and A and C, respectively. Identical results by all labs were 50% no growth, 35.1% Staph. aureus, 6.8% Strep. uberis, 4.1% CNS, 2.7% Coliforms, 1.4% Strep. dysgalactiae.

Identical results considering antimicrobial susceptibility (i.e., susceptible, intermediate, resistant) were provided by all labs for 57.1% of the tested antimicrobials. Lab A and B provided the same results for 67.6% (Kappa = 0.322; $R = 0.432$; $P < 0.001$), Lab B and C for 61.1% (Kappa = 0.257; $R = 0.341$; $P < 0.05$) and Lab A and C for 71.0% (Kappa = 0.308; $R = 0.391$; $P < 0.001$) of the antimicrobials, respectively.

While somatic cell count results differed between labs as well ($P < 0.02$), the correlation was good (i.e., $R = 0.854 - 0.959$; $P < 0.001$).

Conclusions: Our study showed a fair to moderate agreement between laboratories considering pathogen identification, antimicrobial susceptibility and SCC. While our results slightly differ from previous comparisons utilizing spiked milk samples, they implicate that the bacteriological examination of milk samples might have limitations, e.g. if samples are sent by regular mail or without cooling. Considering current discussions on bacterial resistances and antimicrobial residues, further research is warranted to identify confounders that affect bacteriological results and to improve the reliability of results acquired by bacteriological examination.

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Validity of the Delvo Test SPNT® for Detection of Residues of Novel Antimicrobial Substances in Milk

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Objectives: The objectives of this study were to validate the Delvo test SPNT® for detection of residues of polyether ionophores in milk. The use of the Delvo test SPNT® is in detection of factors inhibiting the growth of the Bacillus stearothermophilus var calidolactis in the milk. Almost 80% of antibiotic residues in milk can be traced back to mastitis treatment. Systemic and oral treatment may also result in violative residues in milk. As some of the polyether ionophores are used orally, the potential of excretion in milk is present. Additionally, currently these antimicrobials are assessed for their potential use in mastitis treatment.

Materials and Methods: Four polyether ionophores (lasalocid, monensin, narasin and salinomycin) were used as test antimicrobials. Two beta-lactam antimicrobials (amoxicillin and cloxacillin) were used as positive controls. Non-spiked milk was used as negative control. The study was carried out in few steps, preparation of stock solution of the antimicrobials, spiking milk with known concentrations of the antimicrobials of interest and test operation.

Milk used in this study was collected from four individual cows known not to have been treated or fed with antimicrobials at least two weeks prior to the milk collection. A 0.75L portion per cow was comingled (commingled milk).

Stock solutions were prepared following the Clinical and Laboratory Standards Institute guidelines.

The spiked milk samples were prepared daily. Fixed volume (1µL) aliquot of the appropriate working solution at different antimicrobial concentrations was added to 99 µL of milk.

Operation of the Delvo test SP-NT® test was carried out following the manufacturer's recommendations. The end point colour change of all the test was assessed and recorded by a visual colour score from DSM food specialists®. Following the incubation period the colour change observed in the test bed was classified as one of the following categories: 100% yellow, 75% yellow, 50% purple, 75% Purple or 100% purple. A classification of 50% purple or greater was recorded as a positive test result. If discrepancy of more than one category occurred the testing for the appropriate antimicrobial concentration was repeated (n=2 occasions amoxicillin).

Results: No difference between the results obtained in full cream and skim milk sample was detected. Additionally, there was no difference in the results obtained from milk samples from individual cow or comingled milk. No false-negative or false-positive reactions were observed above or below the limit of detection (LOD) for any of the tested antimicrobials.

The Delvo test SP-NT® test was able to detect all four polyether ionophore compounds in milk in relatively low concentrations. The LOD for monensin was 0.25 µg/mL, for salinomycin 0.50 µg/mL, and for lasalocid and narasin was 1.00 µg/mL. Unfortunately, the LOD was 100-folds higher than the only registered MRL in Australia for the lasalocid.

The LOD for amoxicillin 0.003 µg/mL and for cloxacillin was 0.03 µg/mL. The LODs were within the industry standards for the Delvo test SP-NT® test. The LOD for amoxicillin was 3.3 times higher and for the cloxacillin 3 times below the Australian MRLs.

The DMSO was used to dilute the lipophilic and immiscible in water polyether ionophores. Milk was used as media for testing. The DMSO has been shown to have some antimicrobial activity against some microorganisms on its own. The regular monitoring programmes using microbial inhibition tests has also shown that milks from some cows and especially in early lactation of just before an episode of clinical mastitis may hold some unidentified inhibitory substances. Therefore, the DMSO and milk alone and in combination were used as control wells. Fortunately, no test with the DMSO and milk alone or in combination was positive.

Conclusions: The results from this study indicate that the Delvo test SPNT® is capable of detecting all four tested polyether antimicrobials. Unfortunately, the LOD was significantly higher than the registered MRL in Australia.

Ideally, the screening test should detect the potential antimicrobial residues of concern at or below the relevant MRL. Additionally, to protect the consumer, the test should produce minimal proportion of few false-negative results and to protect the producer, national and international trade, minimal proportion of false-positive results. The test performance regarding the false-positive and false-negative results was satisfactory.

Udder Health & Milk Quality

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Characterization of fatty acids in bulk tank milk of dairy herds from Valle Camonica, a mountain zone in Lombardy Region, Italy

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Objectives: Aim of this survey has been to characterize and evaluate saturated (SAT), unsaturated (UNSAT), monounsaturated (MONO), polyunsaturated (POLY) fatty acids by Fourier Transform Infrared spectroscopy (FTIR) of the bulk tank milk collected from a representative sample of dairy herds located in a mountain area in province of Brescia, Lombardy region. The average fatty acid milk composition of 37 grazing dairy herds selected was compared with the detected one of 54 dairy herds feeding with uni-feed in flat lands.

Materials and Methods: Thirty seven dairy herds for 492 lactating cows, mainly Brown Alpine and Red Piebald, were selected on their feeding based mainly on hay, supplementary feed, without silage maize (October-May) and on the grazing in summer period (June-September). These were small size herds (average 12 heads) producing cheeses for self-consumption and/or direct sale. A questionnaire was realized and filled in order to determine the main features of the farms, particularly the feeding composition. Three alpine pastures were visited and followed up during summer mountain grazing. From April to August 2015 refrigerated bulk tank milk samples (BTMS) from all the selected herds were tested before (April-May) and during (June-July) the grazing season twice a month, for a total of 189 samples. Fat, protein, lactose, casein were analyzed by FTIR spectrometry MilkoscanFT6000® (Foss, DK) with management software Foss Integrator®. Calibration curves developed by producer for SAT, UNSAT, MONO, POLY fatty acids were recalibrated and validated with gas chromatography by our laboratory using milk samples from regional herds. Data were processed by univariate analysis using IBM SPSS STATISTICS 22.0.

Results: The mean values for fat, protein and casein detected on 37 dairy herds were, respectively, 4.15 g/mL (SD 0.602), 3.37 g/mL (SD 0.229), 2.64 g/mL (SD 0.185) with a casein/protein ratio of 0.783 (SD 0.007).

SAT, UNSAT, MONO, POLY mean values were, respectively, 2.569 g/100g (SD 0.404), 1.233 g/100g (SD 0.272), 1.129 g/100g (SD 0.248), 0.150 g/100g (SD 0.040).

The class of major frequency for SAT and UNSAT was 2.7 g/100g (1.9-3.65) and 1.2 g/100g (0.95-2.45).

Comparing the results on milk produced in tie-stalls located in valley (April-May) vs summer pastures (June-July) mean values of fat increased from 4.01 to 4.35/100g, despite of the adaptation to the pasture, probably compensated by a better animal welfare.

It is interesting to remark that UNSAT-SAT mean ratio on 189 BTMS increased from 0.42 to 0.57 g/100g ($p < 0.05$) during the grazing period. MONO and POLY mean values increased, respectively, from 1.00 to 1.28 g/100g and from 0.12 to 0.17 g/100g ($p < 0.05$).

The 37 dairy herds grazing dairy herds were also compared with 54 intensive dairy herds located in flat area during the same period, in order to evaluate variation in fatty acid composition, depending on the type of feeding. Fat mean value was slightly different (4.01 vs 3.96g/100g), while UNSAT-SAT mean ratio, 0.57 vs 0.44 g/100 g, resulted quite different ($p < 0.05$).

Conclusions: The survey confirms the close positive relationship between the grass feeding on summer alpine pastures and fatty acid composition in the milk, that are significantly higher than in fall-winter season. There is the evidence that milk produced using grazing pastures is characterized by UNSAT-SAT mean ratio higher than dairy herds in flat territory using uni-feed. Particularly, UNSAT-SAT mean ratio can represent an important index to evaluate the milk quality oriented to the cheese production and to qualify the nutritive value of the typical dairy products.

Udder Health & Milk Quality

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New Diagnostic Tool for the Detection and Quantification of Pathogenic Bacteria in Bovine Milk Samples from Dairy Farms in UK

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Objectives: Using a FTA Card milk sample for PCR-RT testing (STARTCHECK®) is a reliable diagnostic tool that uses a new methodology to collect milk samples and detect the major mastitis causative agents. PCR-RT testing (STARTCHECK®) can be used to complement Somatic Cell Count (SCC) and Bacterial culture to monitor mastitis at a herd level(1). This tool (STARTCHECK®) has been used worldwide since 2009 and milk samples from 44 different countries have been analysed. The aim of this study is to show the results obtained in UK dairy farms.

Materials and Methods: A total of 366 Bulk Tank Milk (BTM) and Mastitis Pool samples were collected from different dairy farms in UK. The samples were taken according to the kit (STARTCHECK®) instructions [Impregnating the designated areas (BTM and Mastitis Pool) on the FTA card with 250ul of the respective milk sample](2) and sent by ordinary mail to DIAGNOS (HIPRA) in Amer, Girona, Spain. (Fig. 1).

The samples were processed and tested using the Real-Time Multiplex PCR assay as previously described (2), to detect the presence of *Staphylococcus aureus*, *Escherichia coli*, Coagulase Negative Staphylococci (CNS) and Coliform bacteria. The results were determined as positive or negative based on Cycle threshold (Ct) values, with Ct values below 37 being considered as positive.

Results: All samples collected in the UK using this tool (STARTCHECK®) were analysed in the specialized HIPRA facilities by using a PCR-Real Time test. Results were obtained in a semiquantitative way. and the positiveness to each mastitic pathogen was as follows:

- 98% of the bulk tank milk samples were positive to Coagulase Negative Staphylococcae
- 63% of the bulk tank milk samples were positive samples to *E. coli*
- 13% of the bulk tank milk samples were positive samples to other coliforms
- 22% of the bulk tank milk samples were positive samples to *Staphylococcus aureus*

Conclusions: FTA Card milk sample for PCR-RT testing (STARTCHECK®) is a good diagnostic tool to identify pathogenic bacteria present in milk samples. The high specificity and sensitivity circumvents limitations experienced with bacteriology (1). Compared with worldwide data, it should be noted that the presence of *Staph. aureus* in UK samples (22%) is higher than samples obtained from other countries (6-10%) (2) Further data will be discussed in more detail during the WBC congress.

Udder Health & Milk Quality

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Prevalence And Resistance Trends Of Causative Agents Of Bovine Subclinical Mastitis Over A 10-Year Period In Northwestern Croatia

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Objectives: The aim of this study was to determine causative agents of bovine subclinical mastitis (BSM) over a 10-year period (2001-2010), their prevalence and in vitro resistance to antibiotics. Additionally, we monitored the influence of farm size and management system changes during the observation period on the incidence of BSM and effectiveness of antibiotic therapy.

Materials and Methods: Milk samples for microbiological examination were taken from udder quarters of cows without clinical symptoms of mastitis kept at dairy farms in northwestern Croatia that were positive to California or Zagreb mastitis reagents. Prior to sampling, the first milk steams were discarded and the next were examined with the mastitis reagent. When positive reactions were obtained with either mastitis reagent (colour or density change), teat ends were disinfected with cotton swabs soaked in 70% alcohol and new milk samples were collected. The samples were transported in cooled refrigerators to the laboratory as soon as possible. Microbiological agents and antibiograms were performed at the Croatian Veterinary Institute,

Krizevci, Croatia according to common standard protocols. A total of 426 milk samples from dairy cows were examined for the presence of BSM causative agents.

Results: From the total collected samples (n=426), the prevalence of causative agents of BSM was recorded as follows: 44.70% *Staphylococcus aureus*, 24.70% *Staphylococcus sp.*, 9.64% *Streptococcus dysgalactiae*, 4.47% *Streptococcus agalactiae*, 3.76% *Streptococcus sp.*, 6.82% *Escherichia coli*, 1.88% *Enterobacter sp.*, 0.70% *Pseudomonas aeruginosa*, 2.35% *Bacillus sp.*, 0.47% *Candida sp.* and 0.23% yeasts. The prevalence of streptococcal BSM decreased from 30.77% to 10.77% during the 10-year observation period, while BSM caused by *Staphylococcus spp.* increased from 42.31% to 80.00%. Commercial discs were used to perform the antibiogram with the following antibiotics and chemotherapeutics: amoxicillin/klavulanic acid, ampicillin, cefoperazone, cephalixin, cefquinom, enrofloxacin, kloxacillin, lincomycin, neomycin, novobiocin, penicillin, streptomycin, sulphonamide/trimethoprim and tetracycline. After prolonged use of some of these antibiotics for intramammary treatment of BSM, bacterial resistance developed. Namely, only 7.69% of all bacteria isolated were resistant to amoxicillin and klavulanic acid in the first year of the study (2001), while this increased to 38.46% after 10 years. Resistance to some antibiotics increased from 2001 to 2010 was as follows: cefoperazone (0% vs. 15.38%), cephalixin (0% vs. 40.00%), cefquinom (0% vs. 21.53%) and penicillin (19.23% vs. 49.23%).

Conclusions: It could be concluded that during the 10-year observation period, the incidence of BSM caused by *Staphylococci spp.* was significantly increased, as was the resistance of bacteria to certain antibiotics, as a consequence of their frequent use at larger dairy farms (more than 50 cows per farm) and the easier spread of bacteria within and between larger herds in comparison to the beginning of the study when dairy farms in northwestern Croatia were predominately smaller (1-10 cows per farm).

Udder Health & Milk Quality

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Association between etiology of clinical mastitis and characteristics of dairy cows

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Objectives: The objective of this study was to evaluate the association between etiological profile of clinical mastitis and characteristics of affected cows (average milk yield, stage of lactation and number of lactation) in dairy herds.

Materials and Methods: Milk samples from all clinical mastitis cases were collected aseptically during 12 months from 20 dairy herds, which had an average of 218 (SD ± 325) lactating cows, and submitted to bacteriological culture. The microbiological results were categorized as gram-positive, gram-negative and non-bacterial microorganisms (yeast and *Prothotheca spp.*) named in this study as "other microorganisms". Only positive cultures with isolation of a unique pathogen were evaluated in this study. Mixed cultures (n=16; two microorganisms isolated from the same sample) and contaminated samples (n=113; more than two microorganisms isolated from the same sample) were excluded from the analysis. During the sample collection, data at the cow level as the

average milk yield before the occurrence of clinical case, parity and days in milk (DIM) were recorded. The effect of selected explanatory variables on risk of isolation of etiological groups of clinical mastitis was tested using logistic regression. The explanatory categorical variables used in statistical models were milk yield (≤ 20 , 21 to 35, and >35 L/cow/d), stage of lactation (early: ≤ 100 ; middle: 101 to 200; and, late: >200 DIM), and parity (1, 2, and ≥ 3 lactations). The models contained herd as a random effect to correct for clustering of cows within herds. Odds ratios (OR) were calculated with 95% confidence interval and the statistical significance was defined as $P \leq 0.05$.

Results: A total of 3,703 milk samples were submitted to microbiological culture during the study period and 2,151 cultures had isolation of a unique pathogen. Of this total, 1,515 cases (70.44%) had isolation of gram-positive bacteria, 520 cases (24.17%) of gram-negative bacteria, and 116 cases had isolation of other microorganisms. Cows in early stage of lactation were 1.36 and 1.68 times more likely ($P=0.001$) to present a clinical mastitis caused by gram-negative bacteria than cows in middle and late stages of lactation, respectively. On the other hand, cows in middle and late stages of lactation were 0.74 and 0.72 times more likely ($P=0.02$) to have a clinical mastitis caused by gram-positive bacteria compared to cows in early stage of lactation. Cows in early and middle stages of lactation were 2.66 and 3.00 times more likely ($P=0.006$) to have a clinical mastitis caused by other microorganisms than cows in late stage of lactation. In addition, first-lactation cows were 2.69 and 2.96 times more likely ($P=0.0003$) to present a clinical mastitis caused by other microorganisms compared to cows that were in second and third or greater lactation. There was no association between gram-positive and gram-negative bacteria and parity of affected cows. In addition, there was no association between the causative groups of clinical mastitis and the average milk yield before the occurrence of clinical mastitis in dairy cows.

Conclusions: Gram-negative bacteria are more likely to cause clinical mastitis in early stage of lactation, while gram-positive bacteria are more likely to cause clinical mastitis in the middle and late stages of lactation. In addition, *Prototheca* spp. and yeast are more likely to cause clinical mastitis in primiparous cows and during the early and middle stages of lactation.

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Udder Health & Milk Quality

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Genetic and Economic Analysis for the Relationship between Udder Health and Milk Production Traits in Friesian Cows

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Objectives: The aims of this study were to evaluate the genetic and phenotypic relationship between milk production traits and udder health traits, estimation of genetic parameters for these traits and estimation of the economic milk losses due to bad udder health traits in Friesian cattle raised in a governmental farm in Egypt.

Materials and Methods: The present study used data from the monthly lactation records of Friesian cows belonging to Sakha Animal

Production Research Station, Animal Production Research Institute (APRI), Ministry of Agriculture situated at Kafrelsheikh Governorate in the northern part of Nile Delta of Egypt. Initially, the number of records used was 7,450 monthly lactations involving 784 Friesian cows sired by 89 sires. Excluding some incomplete records and trait limitations (e.i, cows were removed with i) no measurements on yields of milk, fat and protein or with records from <240 , d and >400 , d length of lactation; ii) duplicate termination codes and iii) duplicate records) the final total number of records used in the statistical analyses was 4,752 of 439 Friesian cows sired by 52 sires. Milk production traits : The averages of daily milk yield, monthly milk yield and 305-day milk yield were calculated for each cow. Daily milk yield was calculated as the 305 day milk yield divided by days in milk. Milk samples were taken weekly from each cow for chemical analysis of the milk constituents which included: percentages of fat, protein, lactose, total solids and solid non-fat (SNF). The following milk production traits were studied: 305-day milk yield (305-dMY, Kg), 305-day fat yield (305-dFY, kg) and 305-day protein yield (305-dPY, kg). Somatic cell count (SCC) : SCC was measured by a Fossomatic Cell counter from a sample of the milk collected during the morning milking. The determination of SCC (by thousand cells per millimeter milk) was performed at Dairy Service Unit belonging to APRI, Sakha.

Results: Overall unadjusted means of 305-day milk yield, 305-day fat yield, 305-day protein yield and somatic cell count across lactations were $3,936 \pm 1,044$, 121 ± 54.3 , 90 ± 38.0 kg and 453 ± 218 thousand cell/ml. The coefficients of variation for the same traits were 26.6, 44.8, 42.2 and 48.1%, respectively. Most studied milk production traits were affected negatively, at different levels, in UDHS classes (1 to 9 classes) when compared with the normal udder (0 class). It could be noticed also from Table 2 that 85.1% of observations in this herd (4,045 records from 4,752) had normal udder (0 class). Clinical mastitis represented the highest percentage of UDHS defects (class number 3) with 10.2% of all observations. Meanwhile, the other eight classes (excluding normal udder and clinical mastitis) represented 4.7% of all observations. The highest losses in milk production traits were associated with chronic mastitis treatment (class No. 7) which had the highest negative least square constants (-570 kg for 305 dMY, -9.96 kg for 305 dFY and -9.19 kg for 305 dPY) followed by clinical mastitis (class No. 3) with the 2nd rank of the negative least squares constants (-193 kg for 305-dMY, -5.49 kg for 305-dFY and -3.45 kg for 305-dPY) when compared with the other UDHS defects. The majority of observations (85.1%) were with normal udder (0 class) as reported in (Table 2). The percentages of udders with one quarter infected were 0.84 and 0.95% for right and left fore quarters (classes 1 and 2), respectively and 2.02 and 3.14% for right and left hind quarters (classes 3 and 4), respectively.

Conclusions: it can be concluded that the economic losses from mastitis and high SCC are considerable. Heritability estimate for mastitis is low (0.14). There is an unfavorable genetic correlation between udder health and milk production traits and this emphasizes the need to include mastitis resistance in the breeding goal. Accuracy of selection can be increased by combining information on MAST and SCC, which are rather strongly genetically correlated (0.85) the high genetic correlation between SCC and clinical mastitis suggest that the selection for lower SCC would help to reduce or eliminate the undesirable correlated responses of clinical mastitis.

Udder Health & Milk Quality

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Evaluation of a Chromogenic On-farm Culture System for Fast Identification of Milk Pathogens Associated with Clinical Mastitis in Dairy Cows

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Objectives: Objectives were to evaluate the use of a selective chromogenic culture system designed for identification of specific mastitis pathogens: Staphylococci, Streptococci, and Gram-negative bacteria, constituted by a single plate containing three selective chromogenic media (Accumast® - FERA Animal Health LCC, Ithaca, NY). Predictive values of this method were evaluated in a first set of samples based on aerobic culture results from an official diagnostic laboratory used as gold standard and on a second set of samples through molecular identification of cultured pathogens using 16S rRNA sequencing as gold standard.

Materials and Methods: A total of 538 mastitic milk samples was collected by trained farm personnel following recommendations of the National Mastitis Council and plated onto Accumast® using a sterile cotton swab. Plates were incubated at 37°C for 24 h and read on-farm according to the flowchart provided. Immediately after collection and plating on-farm, milk samples were refrigerated and transported to the QMPS laboratory (Cornell University, Ithaca, NY). Milk samples were cultured following standard procedures and microbiological culture results were used as gold standard. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were calculated comparing results from on-farm culture and reference laboratory results using the same milk samples. Accuracy was calculated by dividing the number of true positives and true negatives by the total number of tests. The agreement between reference laboratory and on-farm culture was assessed through simple Cohen's kappa coefficient (κ) using the FREQ procedure of SAS.

A second set of samples underwent 16S rRNA sequencing. Plates were read at the farm and those with bacterial growth were transported at room temperature to the laboratory for bacterial isolation, DNA extraction, amplification and sequencing of the 16S rRNA gene using the Illumina platform. Reads were aligned to the Illumina-curated version of Greengenes database for genus-level classification. The PPV and agreement between sequencing and Accumast® were assessed as previously described using 16S rRNA results as a gold standard.

Results: In the present study, the most prevalent pathogens in mastitic milk according to results from QMPS laboratory culture were *S. uberis* (24.9%), *Streptococcus* sp. (10.4%), and *E. coli* (9.1%). Aerobic culture resulting in no bacterial growth accounted for 31.2% of samples cultured, and the prevalence of *S. aureus* was 1.3%. The use of on-farm culture resulted in high overall sensitivity, specificity, PPV, NPV, and accuracy when compared to standard microbiological culture for identification of mastitis pathogens. The Se, Sp, PPV, NPV, accuracy, and κ were 81.6%, 98.9%, 92.5%, 97%, 96.4% and 84.7% for the identification of Gram-negative bacteria, 100%, 99.8%, 87.5%, 100%, 99.8%, and 93.2% for the identification of *S. aureus*, 70%, 95%, 45.7%, 98.1%, 93.6%, and 52% for the identification of *Staphylococcus* sp. and 90%, 92.9%, 91.8%, 91.2%, 91.5%, and 82.9% for the identification *Streptococcus* sp. All P-values associated with kappa coefficients were <0.001 .

The overall PPV and κ of Accumast® when compared to 16S rRNA sequencing was 96.7% and 85.7% for *Escherichia*, 100% and 100% for *Enterococcus*, 100% and 95.3% for Other Gram-negatives., 88.2% and 93.2% for *Staphylococcus* sp., and 95.0% and 91.8% for *Streptococcus* sp.

Conclusions: Color based identification of bacteria enables individuals with limited microbiological training to easily interpret culture results. The culture system evaluated here provides a unique approach for on-farm identification of mastitis associated pathogens, providing the basis for selective antimicrobial therapy of mastitic cows according to the pathogen causing the infection. Overall accuracy was 84.9%, Sensitivity was 82.3%, and Specificity was 89.9% for identifying mastitis pathogens compared with results from standard laboratory. Test characteristics of the method were confirmed through 16S rRNA gene sequencing.

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Comparison of PCRs to culture results in detection of Mycoplasma spp. from milk samples from South Australia

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Objectives: The objectives of this study were to estimate and compare the detection capability of a novel PCR with traditional bacteriological culture method of *Mycoplasma* spp from cow milk samples collected from a single dairy farm in Mount Gambier, South Australia, Australia. The farm had a high incidence of clinical mastitis but also many cows with a high SCC and the treatment with antimicrobials was of little value.

Materials and Methods: A total of 366 milk samples at cow level were used. Samples originated from cows aged 2-10 years from the hospital mob of the farm with 2400 milking cows. Approximately 5 mL of milk per cow were placed immediately for enrichment into a *Mycoplasma*-selective media broth. At the lab, samples were incubated anaerobically with 10% CO₂ at 37°C. Seven days later, the samples were plated on *Mycoplasma*-selective agar. The presence of *Mycoplasma* colonies was detected using light microscopy at 10x magnification. The DNA was extracted using original milk samples and a PCR technique was used to detect the presence of *Mycoplasma* spp. using specific primers for *Mycoplasma* (16S RNA).

Results of both procedures were compared using kappa agreement. Test characteristics of each test were also assessed. Unfortunately, no gold standard for detecting *Mycoplasma* spp. exists. Therefore, our 'gold standard' was any positive sample detected by either procedure.

Results: *Mycoplasma* species was isolated from 184/366 (50.2%) of mastitis milk samples using the microscopic bacterial culture method. These samples were considered positive when there is a growing of at least one *Mycoplasma* colony in the plate. *Mycoplasma* spp. was identified more frequently using PCR technique with 219/366 (59.8%). The test characteristics of the culture results were Sp= ; Se= ; ACC= while for the PCR, it were Sp= Se= , ACC= .

THE PCR RESULTS WILL BE AVAILABLE IN LATE MARCH TO EARLY APRIL 2016 THE SE, SP AND ACC OF EACH TEST WILL BE ESTIMATED AFTER PCR RESULT BECOME AVAILABLE

Conclusions: The results of this study indicate the significant difference of the detection of *Mycoplasma* mastitis using the novel PCR technique in comparison with the traditional bacterial culture method. In addition, the accuracy of PCR detection technique particularly for the frozen milk samples may have a great value for veterinarians and farmers dealing with difficult to cure mastitis cases.

Comments: The study is on-going. The culture results are available. The PCR results should be available by late March to early April 2016.

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Case Report of the Utilization of a Polyvalent Vaccine for Mastitis in the Dry Period and the Milk Quality and Fertility outcomes

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Objectives: Even though mastitis control programs are frequently used in dairy production, mastitis are still one of the most frequently occurring and costly diseases in modern dairy farms (Halasa et al., 2007).

Mastitis monovalent vaccines are widely used in some countries showing significant results in the reduction of severity of clinical signs and duration of infection of environmental mastitis (Wilson et al., 2009). The goal of this study was to evaluate the impact in milk quality, fertility and economic outcome of a polyvalent vaccination protocol for mastitis (Startvac®, Hipra, Barcelona).

Materials and Methods: The study was held in a commercial dairy farm of 450 lactating cows on average, situated in the northwest Portugal. The animals were allocated to each group in an alternating form when entering the dry period (157 in the control group and 159 in the vaccinated group). The first dose was administered 8 weeks prior to calving and the rappel applied 4 weeks later. The period of vaccination occurred between October 2013 and July 2014. Production data, somatic cell count (SCC) and fertility data were compiled in an Excel file for posterior statistical analysis. Animals that were culled or died for other reasons than mastitis were excluded from the final analysis. The average value of the milk production difference between the groups was calculated based on the average price of milk sold during the period of study.

Results: The results obtained showed significant differences between the groups. Concerning the SCC a significant reduction in the average values of the three months for multiparous was observed being more visible on the second month of lactation (342×10^3 cells/ml in control group and 86×10^3 cells/ml in vaccinated group; $p < 0.01$), potentiating milk production. There was also a positive difference of milk production on the three months favouring the vaccinated group although only statistically significant on the second month of test day of the multiparous cows (2.35 L; $p < 0.05$).

In effect, the vaccinated group registered less 52 mastitis cases thus explaining the raise in milk production when compared to the control group.

Concerning the fertility indexes the calving-first insemination interval was 11.5 shorter for multiparous ($p < 0.01$) contributing to the decrease of the calving-conception interval.

On the economic analysis, the vaccine showed a gain of 47.10 € on the first three months of lactation on the multiparous of the vaccinated group. This value was of 21.15€ when just evaluating the second month when the difference was more significant.

Conclusions: This study showed that the utilization of this polyvalent vaccine for mastitis reduced the somatic cell count and improved the milk production of the vaccinated cows. Additionally, less clinical cases were registered during the period of analysis in the vaccinated group. Finally,

the immunized group performed better in terms of fertility indexes, namely, on the reduction of the interval from calving to conception. These results showed that the utilization of this polyvalent mastitis vaccine is an important additional tool to include in the prevention mastitis programs.

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In-feed inclusion of clinoptilolite reduces milk aflatoxin M1 concentration in dairy cattle

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Objectives: Clinoptilolite is a natural zeolite with high adsorption capacity for polar mycotoxins. Its efficacy in ameliorating the toxic effects of aflatoxicosis has been proven in monogastric animals, but no evidence exists for ruminants. The aim of this study was to evaluate, under field conditions, whether the dietary administration of clinoptilolite in dairy cows reduces aflatoxin M1 (AFM₁) concentration in bulk-tank milk, when AFM₁ concentrations were higher than or close to the European maximum allowed residual level ($0.05 \mu\text{g AFM}_1/\text{kg}$ of milk) and investigate whether the particle size could affect clinoptilolite's aflatoxin binding efficacy.

Materials and Methods: The study was conducted in 15 commercial Greek dairy herds that had high concentrations of AFM₁ in bulk tank milk (close to or higher than $0.05 \mu\text{g AFM}_1/\text{kg}$ of milk), as detected at the routine analyses by the dairy industries collecting the milk, using their standard methods of determination. The criteria for the selection of the farms were: a) the willingness of the farmers to use only clinoptilolite as mycotoxin binder without making any other changes to the rations offered to the animals during the experimental period, and b) the agreement of the milk industries to determine the AFM₁ concentrations in the delivered bulk tank milk at fixed time-points. The study lasted 7 days. On Day 0, the bulk tank milk AFM₁ concentration was determined. For the next seven days (Days 1 to 7), clinoptilolite was added in the total mixed rations of all farms at the level of 200 g per animal, per day. The bulk tank milk AFM₁ was also determined at the delivered milk produced on Day 7 of the experiment. According to the size of zeolite used, the farms were allocated into two groups. The first group (SC) was consisted of 9 farms that used the $< 0.15\text{mm}$ particle size and the second one (LC) of the other 6 farms that used the $< 0.8\text{mm}$ size. All statistical analyses were done with SPSS® version 21.

Results: The dietary administration of clinoptilolite for seven consecutive days significantly reduced milk AFM₁ concentrations in all farms tested at an average rate of 56.2% (SD: 15.11). The mean milk AFM₁ concentration recorded on Day 7 was significantly lower compared to that of Day 0 (mean \pm SE: $0.078 \pm 0.0074 \mu\text{g/kg}$ and $0.036 \pm 0.0061 \mu\text{g/kg}$ for Days 0 and 7, respectively, $P < 0.001$). In farms of the LC group the reduction of milk AFM₁ concentration was significantly higher than in farms of HC group (mean \pm SE: $0.046 \pm 0.0074 \mu\text{g/kg}$ and $0.036 \pm 0.0061 \mu\text{g/kg}$ for groups LC and HC, respectively, $P = 0.002$). As indicated by the Pearson correlation, there was a significant and strong linear correlation among the milk AFM₁ concentrations on Days 0 and 7 ($R = 0.95$, $P < 0.001$). According to the results of regression analysis, the equation yielding the predicting value of milk AFM₁ concentration after the administration of clinoptilolite (Day 7) was:

$$\text{AFM}_{1F} = 0.781 \times \text{AFM}_{10} - 0.023, (r^2 = 0.899, P < 0.001),$$

where AFM_{1F} represents the milk AFM₁ concentration on Day 7 and AFM₁₀ the milk AFM₁ concentration on Day 0.

Conclusions: Dietary administration of clinoptilolite, especially of smallest particle size, can effectively reduce milk AFM₁ concentration in dairy cattle. Taking into consideration that the long-term dietary supplementation of clinoptilolite is safe and has beneficial effects on the health status and productivity of dairy cattle, clinoptilolite feeding at the rate of 200 g per cow per day can be used as a preventive measure for the amelioration of the risks associated with the presence of aflatoxins in the milk of dairy cows.

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The Overall, Quarters and Herd Size Prevalence of Mastitis in Dairy Buffaloes

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Objectives: Dairy farming in Pakistan is primarily fragmented into traditional rural, progressive/semi-commercial rural, peri-urban and commercial/corporate farming. However, the traditional rural farming is the largest segment among all. The majority of dairy farmers are smallholders with substantial number of landless farmers. The cross sectional study was carried out to investigate the prevalence of clinical and subclinical mastitis in dairy buffaloes.

Materials and Methods: The present study was conducted in two districts Lahore and Bhimber. Total sample size for this survey was consisted of 1,036 lactating buffaloes. The screening of lactating buffaloes was performed with the California Mastitis Test. All the collected data through questionnaire was entered into an Excel sheet of Microsoft Office Excel (2003) and transferred for analysis to R software and SPSS version 16.0. Firstly, all the data were labelled into sub heading and analyzed by using descriptive statistics for the frequency and cross tabulation tables.

Results: The overall prevalence of mastitis was recorded at 49%. Among mastitis cases the prevalence of clinical mastitis was 10.2% (106/1036 animals) as a whole. However, the prevalence of clinical mastitis in district Lahore was 11.8% (71/598 animals) whereas it was 8% (35/438 animals) in district Bhimber. The overall prevalence of subclinical mastitis was 38.8% (402/1036). The district wise prevalence of subclinical mastitis was 43.6% (261/598 animals) and 32.2% (141/438 animals) in the districts of Lahore and Bhimber, respectively. The quarter level prevalence was recorded 16.20% (673/4144 quarters from 1036 animals). The district wise prevalence of mastitis at quarter level was recorded 18.17% (449/2392 quarters) in a district Lahore while, it was 12.87% (224/1752 quarters) in district Bhimber. The overall prevalence of mastitis on the basis of herds size was 41/102 (40.2%), 186/400 (46.5%) and 281/534 (52.6%) in small, medium and large herds, respectively.

Conclusions: Mastitis has great economic losses in the dairy industry and it can be minimized by improving management and milking practices. it can be concluded from the present study, the prevalence of subclinical mastitis is higher then clinical mastitis. the prevalence of mastitis was higher in hind quarters then fore-quarters. The prevalence of mastitis was increased as the herd size is increased.

Udder Health & Milk Quality

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Using deviation patterns of in-line milk conductivity to automatically detect mastitis caused by specific pathogens during milking

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Objectives: The objective of this study was to determine whether there are differences in milk conductivity for mastitis caused by different pathogens, to allow accurate early detection of mastitis during milking. This will allow to treat cows promptly and accurately allowing the cows the best chance for fastest recovery.

Materials and Methods: Milk meters equipped with conductivity probes (Afimilk, Israel) installed on a large commercial farm in Spain were used for data collection; every cow, every milking. Cows identified with mastitis by clinical signs (abnormal milk and/or swollen quarter) were evaluated as to the milk conductivity level (mmHO) on the day of mastitis diagnosis, as well as the deviation of that level compared to the average conductivity of the same milking shift during the previous 10 days for each sepcific cow. A milk sample was collected before treatment and submitted to a reference laboratory for identification of isolated bacteria.

Results: A total of 92 samples were included in the evaluation. On average, cows with mastitis showed a mean conductivity of 13.0±1.4 mmHO compared to 10.1±0.5 mmHO throughout the entire lactation of the whole herd (P<0.001). Cows with mastitis showed a 19% deviation from the average of the previous 10 days. Variations were evident depending on isolated bacteria. Most notably, cows infected with E. coli had higher conductivity deviation (26%) than if infected with other bacteria (19%, P<0.001), while the average conductivity not different between the groups (P=0.745). No other statistically significant differences were observed, although numerical differences were evident between Gram + and Gram - bacteria.

Conclusions: Instead of looking for a fixed conductivity breakpoint in milk conductivity to diagnose a cow with mastitis, milk conductivity can be effectively used to detect mastitis caused by E. coli versus other organisms due to different deviation patterns from the average conductivity of the previous 10 days of each cow.

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Population structure of infective *Streptococcus uberis* strains in two farms in northern Portugal

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Objectives: Bovine mastitis leads to significant economic losses worldwide. Importance of *Streptococcus uberis* (SU) has increased in udder health. Currently two distinct epidemiologic patterns of SU are

recognized: environmental or contagious. Different control strategies are needed to control these patterns. In this work, persistent infected cows from two dairy herds with high SU infection were followed two consecutive years. Purpose: to isolate, characterize and search for known virulence-associated genes of *S. uberis* in quarter milk. Main objective: to disclose prevailing genotypes of SU focusing on persistency, pathogenicity and population structure.

Materials and Methods: Two dairy herds located in Northern Portugal (Barcelos – herd 1; Maia – herd 2) were visited in two consecutive years (2013 and 2014). In the first visit all the lactating cows were clinically evaluated and California Mastitis Test was carried out at quarter level. Milk samples from each quarter were collected for quantification of SCC (somatic cell count) and bacteriological culture. Preliminary identification of bacterial isolates was performed using the VITEK 2 system and *S. uberis* was identified (bioMérieux, Durham, NC). The infected cows were monitored in the following months and milk from all quarters was screened for the presence of *S. uberis*; CMT and SCC were always done.

Dot blot hybridization assays, coupled with automatic image analysis software, were carried out using 19 DNA probes to characterize the genotypes of *S. uberis* isolates: four taxa-specific probes for *S. uberis* species confirmation; two probes targeting the fructose operon and two the nisin operon; seven probes designed for genes associated to increased virulence and three markers to assess antibiotics resistance.

Multilocus sequence analysis (MLSA) of 30 *S. uberis* isolates was carried out using three housekeeping genes (*ddl*, *gki* and *tdk*). The sequences were concatenated and aligned with the sequences for the same genes of 987 *S. uberis* isolates available at the PubMLST online database. A Neighbor-Joining phylogenetic tree was built to determine the population structure of the *S. uberis* isolates used in this work.

Results: A total of 44 *S. uberis* isolates were obtained from 14 cows (4 from herd 1 and 10 from herd 2) with recurring mastitis infections. After milk testing, while 12 cows showed persistent signs of subclinical mastitis for the duration of the study, two cows presented clinical mastitis.

Dot blot results with the taxa-specific probes confirmed the identity of all isolates as *S. uberis*. Overall, 7 different genotypes were identified in the two herds studied, which had two distinctive profiles. While in herd 1 no prevalent genotype was found, the 30 *S. uberis* isolates from herd 2 had a clearly dominant genotype. The probes addressing antibiotics resistance showed a greater variation amongst isolates. In fact, while the *linB* gene (lincosamide resistance) and *tetS* (tetracycline resistance) were present in 32 *S. uberis* isolates, the *ermB* gene (erythromycin resistance) was present in only 5 isolates.

MLSA characterization showed that these *S. uberis* belong to seven clusters. While in herd 1 a high diversity in *S. uberis* lineages was found, herd 2 was dominantly colonized by the same lineage. Additionally, it was shown that, regardless the herd, recurring infections in 11 from 14 cows were caused by same MLSA cluster. Concerning the two cows with clinical mastitis, while showing different clinical evolution, both remained infected with the same strain for more than five months.

Conclusions: The results obtained in this work suggested distinctive *S. uberis* population structure for the two farms. While in the Herd 1 *S. uberis* were indicative of an environmental pathogen, in Herd 2 the high incidence of a specific *S. uberis* genotype suggests a profile of an infectious pathogen. A transmission pattern was identified in herd 2 and a persistent not transmissible pattern in herd 1; although the two farms are in an intensive system, the cows in herd 1 are allowed to graze outdoors, while in herd 2 they are mostly confined indoors (free stall).

Comments: The authors acknowledge the contribution of Segalab, Laboratório de Sanidade Animal SA the providing the opportunity of following the herds and obtaining the SU strains.

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Seasonal variation in milk composition in five dairy herds

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Objectives: Milk composition may be a useful tool for early detection of nutritional and health problems in a dairy herd. Seasonal variation in milk components is a recognised phenomenon. The results of weekly bulk milk samples during one year period were analysed.

Materials and Methods: Bulk milk samples from five dairy farms with (25-190) dairy cows were taken weekly during one year period. Contents of milk fat, milk protein, lactose, and somatic cell count were measured. Concentration of urea, sodium, potassium, chloride and activity of enzyme lactate dehydrogenase (LDH) was measured in milk serum samples with biochemical analyser Cobas Mira (Roche). Nutrition, health and reproductive status of dairy cows in herds were followed during investigated period. Statistical analysis was performed with programme SPSS (Ver. 22).

Results: The milk fat content was between 4.19±0.18% and 3.63±0.14 with the lowest value in July. The lowest value of milk protein was in June (3.18±0.09%) and the highest values were found from November to February. Variation in lactose content was insignificant. The lowest concentration of urea (3.17±0.88 mmol/L) was measured in April and higher concentrations from June till October with peak in August (4.50±1.18 mmol/L). The concentration of sodium varied between 23.11±2.97 mmol/L in April and 27.31±9.04 mmol/L in June. The concentration of potassium varied between 35.59±1.7 mmol/L in May and 40.35±2.86 mmol/L in November. The concentration of chloride oscillated between 38.85±2.32 mmol/L in February and 43.38±2.53 mmol/L in November. The highest activity of LDH was measured in December (130.59±11.59 U/L) and the lowest activity was found in July (103.93±19.81 U/L). The somatic cell count varied between 258±82 x 10³/ml in October and 384±97 x 10³/ml in May.

Conclusions: Variations in milk composition between herds were observed which were partly related to nutrition and season and partly to the udder health. Detailed analysis of single cows milk composition contributing to these data is planned.

Udder Health & Milk Quality

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Sensitivity And Specificity Of Scc In Quarter Milk Samples To Diagnose Intramammary Infections

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Objectives: Culture results of duplicate quarter milk culture samples have been a common way to determine the infection status of the mammary gland in teat disinfectant clinical trials. In recent years, field trials have evaluated the efficacy of teat disinfectants using a novel

approach that considers a somatic cell count (SCC) threshold to make a culture decision, and to determine intra-mammary infections (IMI). The aim of this study was to evaluate the sensitivity (Se) and specificity (Sp) of SCC as a potential qualifier for milk samples to be cultured, and to test positive for an IMI with mastitis pathogens in clinical trials.

Materials and Methods: Data included cow information, SCC and bacteriological results from the first sampling of ten clinical trials. As part of the trial protocol, first sampling required that all milk samples had SCC and microbiological status information. In total, sterile milk samples of 8,859 quarters from 2,255 cows were analyzed. Trials were conducted in dairy herds located in USA, Canada and Colombia between February 2009 and July 2014. The SCC value from each milk sample was determined using an automated SCC reader. In addition, each milk sample was submitted for aerobic culture following standard procedures (NMC, 1999). The Se, Sp, positive predictive value (PPV) and negative predictive value (NPV) were calculated as described by Dohoo et al. (2009).

Results: Out of the total milk samples cultured, only 27% showed bacterial growth. The most common pathogens were *Staphylococcus* spp (CNS), *Staph. aureus* and *Cor. bovis*. A total of 1,869 (21%) samples had their SCC value above the thresholds set. The receiver operating characteristic (ROC) curve was made to determine the ability of SCC to classify correctly positive quarters, the area under curve was 0.66.

The Se and Sp varied depending on the bacterial species and the cow's lactation number. The overall Se and Sp of SCC to predict milk samples to be cultured was 37.8% (95% CI: 35% - 39%) and 85.3% (95% CI: 84% - 86%), respectively. Both parameters for each bacterium isolated were also calculated. The PPV and NPV of SCC thresholds for all pathogens were 49% (95% CI: 47% - 51%) and 78% (95% CI: 77%>79%), respectively.

The Se according to the isolated pathogen was high when the milk culture was positive for major pathogens (82%; 95% CI: 78% - 86%), such as *Staph. aureus*, *Streptococcus agalactiae* and *Strep. uberis*. However, the Se for minor pathogens was low (29%; 95% CI: 27% - 32%), which would be expected since minor pathogens are normally associated with slight increases in SCC (Schepers et al. 1997).

Conclusions: The new approach in which a threshold of SCC is used as an initial screening test is effective for determining true IMI in clinical trials, mostly caused by major pathogens. The relevance of identifying minor pathogens needs further research. Using this approach, the cost associated with the analyses in traditional trials to test teat disinfectants might be reduced.

Udder Health & Milk Quality

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Prevalence and Management Practices associated with Bulk Tank Milk Prevalence of *Staphylococcus Aureus* and Coliforms in Azorean Dairy Herds

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Objectives: The aims of the present study were: 1) to determine the apparent prevalence of some of the main microorganisms that cause mastitis - *Staphylococcus aureus*, *Escherichia coli* and other coliforms (COL) from bulk tank milk (BTM) samples of dairy farms on

the island of São Miguel- Azores, and; 2) to evaluate the management milking practices associated with these pathogens prevalence, and consequently, the ultimate goal was to emphasize the indication, in Azorean dairy farms, for the use of the first vaccine registered by EMA (European Medicine Agency) for the prevention of mastitis caused by these pathogens (STARTVAC®, HIPRA, Spain).

Materials and Methods: In July, 2014, 100 dairy herds considering a total of 6065 adult cows were used from all regions of San Miguel. These animals represented 11.7% (51.684) of whole adults dairy cows on pasture from 5.5% (1833) herds, according data officially reported in São Miguel Island.

A total of 100 BTM samples from the 100 selected herds were collected using a qPCR commercial sampling kit (Startcheck®, HIPRA, Spain), according the manufacturer instructions, in order to test the presence of *S. aureus*, *E. coli* and COL. A qPCR positive result was recorded when cycle threshold values were ≤ 37 and a sigmoidal amplification plot was obtained. Simultaneously, a questionnaire on management practices was carried out according to 5 main groups: 1) the hygiene of the milking procedures, 2) milking machine and tank 3) diagnosis and treatment of mastitis, 4) drying off, and 5) management of calves. A logistic regression analysis was used in order to evaluate the effect of management practices on pathogens BTM prevalence.

Results: *S. aureus*, *E. coli* and COL were detected in 59%, 75% and 35% of BTM, respectively. Of the BTM positive samples, 79.7 % (47/59) were concomitantly positive for *S. aureus* and *E. coli*, and 42.4 % (25/59) to *S. aureus* and COL. *E. coli* and COL were detected simultaneously in 38.7 % (29/75) of the samples. On herds without routine use of gloves and pre-dipping during milking, or not using hot water for cleaning the milking utensils, there was at least 2.8 more chance (P ≤ 0.05) to present *S. aureus* or COL contamination on BTM. Herds without cleanliness practices of udder, teats or tail, milking mastitic and healthy cows together, and lacking treatment records were more affected (P ≤ 0.05) by the presence of *S. aureus* on BTM. The use of mobile (unrefrigerated) BTM also favored (odds ratio = 3.7; P ≤ 0.01) the detection of COL on BTM.

Conclusions: We have presented baseline figures for major contagious and environmental pathogens in BTM samples from Azorean dairy herds and evidenced that some milking management practices represent risk factors for the detection of these pathogens in BTM. These results should be considered in the design of mastitis prevention and control plans for the control of milk quality and suggest that vaccination for the prevention of mastitis can be a real asset as an additional tool in a mastitis control plan in the Azorean dairy herds.

Udder Health & Milk Quality

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Early Administration of an Internal Teat Sealant to Maiden Dairy Heifers

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Objectives: Precalving administration of internal teat sealant (ITS) to maiden dairy heifers is a widespread practice in NZ and it is mostly performed by veterinarians and trained technicians. The purpose of this study was to assess whether extending the sealing-calving interval, by starting earlier in relation to calving, affects the risk of clinical mastitis (CM) between sealing and calving (to assess the risk of iatrogenic mastitis) or in the first 30 days of lactation (to assess efficacy). If the

interval can be extended without compromising safety or efficacy, veterinary clinics could start heifer ITS administration earlier and better meet demand.

Materials and Methods: All heifers that had ITS (Teatseal®, Zoetis NZ) administered by the clinic (Veterinary Centre Oamaru, NZ) ITS team through a specialised trailer over the winter of 2014 were eligible for enrolment in this observational study. Farms were excluded if the farm did not use the MINDA data management program (LIC NZ) or if the farm records were not deemed to be accurate. Heifers were excluded if all four teats were not sealed or no calving date was recorded. Each farm's heifers were sealed on a single calendar day. CM data were captured between sealing and 31st October 2014. A CM case was defined as any case of CM identified and treated by farm staff according to each farm's standard practice. As date of sealing, and therefore sealing-calving interval, are clustered data (by farm), a mixed effects logistic regression was run with farm as a random effect. The data were analysed in two ways. Firstly, mixed effects logistic regression was used to assess the effect of sealing-calving interval on the risk of CM pre-calving and in the first 30 days post-calving. Secondly, heifers were dichotomised around a time point of four weeks prior to planned start of calving into "early" (before four weeks prior to PSC) and "label" (the remainder) groups, and the same risks were compared by mixed effects logistic regression.

Results: 7166 heifers from 31 farms met the inclusion criteria. Heifer ITS administration was performed between 9th May and 11th July 2014 (range 63 days). Sealing-calving interval ranged from 0 to 145 days (mean 53, median 51). 17 cases of pre-calving CM (0.2%) and 370 cases of CM between calving and 30 days postpartum (5.2%) were reported. The median and mean time to CM in the first 30 days postpartum were 3 and 6 days respectively. Heifers with a longer sealing-calving interval had a numerically lower risk of CM in the first 30 days postpartum but this was not statistically significant (OR = 1.00 (0.99 - 1.01), p= 0.58). Heifers in the "early" and "label" groups were on average sealed for 65 days and 35 days respectively. Heifers in the "early" group had a numerically lower risk of CM in the first 30 days postpartum but this was not statistically significant (OR = 0.80 (0.35 -1.79), p=0.58.) There were insufficient cases of pre-calving CM to draw conclusions about the effect of sealing-calving interval on precalving CM risk. The ITS administration technicians found no difference in ease of administration or proportion of blind quarters with respect to timing of administration.

Conclusions: Commencing ITS administration of NZ spring calving heifers from early May did not increase the risk of CM in the first 30 days of lactation. There was very little CM between sealing and calving, making it difficult to draw any firm conclusions about the risk of precalving mastitis when administering ITS early. However, the fact that the incidence of pre calving mastitis in heifers sealed early was low per se supports the safety of this practice. A limitation of this study is that it is observational which resulted in much of the variation in sealing to calving time explained by farm, as all heifers on a farm were sealed on one day.

Udder Health & Milk Quality

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Mastitis Vaccination using a Commercial Polyvalent Vaccine or a Herd-Specific Staphylococcus Aureus Vaccine – Results of a Controlled Field Trial

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Objectives: Mastitis caused by staphylococci is counted among the most important infectious diseases in dairy cows worldwide. More than 80% of all antibiotics used in dairy farming are applied for the treatment or metaphylaxis of mastitis. Frequently, antibiotic mastitis therapies are not of satisfactory success and can promote the selection of antibiotic-resistant bacteria. Thus, the objective of this study was to reduce the number of mastitis treatments during lactation and to decrease the proportion of cows with elevated individual cow somatic cell counts (ICSCC) at time of dairy herd improvement tests (DHITs) by mastitis vaccination.

Materials and Methods: Motivation of this field trial was an elevated bulk milk somatic cell count (BMSCC) and the detection of *S. aureus* as predominant mastitis causing pathogen in a Saxon dairy herd. The study was designed as a prospective, randomized, controlled and partially blinded parallel group comparison. Deliberately, accompanying procedures were neglected especially as housing, feeding, and milking hygiene were already at a high level.

According to the results of cytobacteriological investigations of quarter milk samples one week before dry cow therapy and including data of preceded DHITs, cows were stratified into the status groups (SG) 2-4 according to their udder health. Heifers were classified as SG 1. Heifers (n=181) and cows (n=416) were randomized within their SG to one of the following vaccine groups (VG): Startvac® (HIPRA Deutschland GmbH) (VG SV), Bestvac® Rind Mastitis (containing herd-specific *S. aureus*-strains; IDT Biologika GmbH) (VG BV) and unvaccinated control (VG Co). On average, the first vaccination was performed at 223rd and the second vaccination at 247th day of pregnancy, and finally, the third vaccination at 53rd day in milk (DIM).

Statistical analysis of the common logarithmized ICSCCs (ICSCC_{log}) and milk yield of DHITs 1-4 was performed. Furthermore, ICSCCs of the DHITs 1-4 were categorized regarding to the categories ICSCC<200000/ml, 200000/ml≤ICSCC<700000/ml and ICSCC≥700000/ml and the distribution was analyzed statistically. Finally, the mastitis therapy frequencies at quarter and cow level as well as the duration of therapies were documented until 305th DIM.

Results: The DIM of the cows within the several groups did not differ significantly (mean: DHIT 1 - 34 DIM, DHIT 2 - 68 DIM, DHIT 3 - 101 DIM, DHIT 4 - 135 DIM) (p=0,126 to p=0,867). Considering the means of milk yield, no significant difference was detected between the VG within SG 1-3 at the four DHIT days (p=0,168 to p=0,919). However, at DHIT 1 it was significant within SG 4 that milk yield of VG SV was lower than in the compared groups (p=0,046). Statistical analysis of ICSCC_{log} of DHITs 1-4 revealed no significant differences of the VG within their SG (p=0,113 to p=0,909). The distributions of the ICSCC of the VG within their SG regarding to the categories ICSCC<200000/ml, 200000/ml≤ICSCC<700000/ml and ICSCC≥700000/ml did not differ significantly (p=0,127 to p=0,843).

Due to the small amount of clinical mastitis data only a descriptive analysis was conducted. There were no obvious differences in the incidence of clinical mastitis and duration of a necessary mastitis therapy, respectively, between the VG within their SG.

Conclusions: Since positive effects concerning the SCC were absent in the own trial as well as in the studies of Bradley et al. (2015) (Startvac®) and Hoedemaker et al. (2001) (herd-specific *S. aureus* vaccine), mastitis vaccines against staphylococci are not a suitable tool to decrease the ICSCCs and, thus, to reduce BMSCC within an appropriate period of time on farms with good udder health management and high hygiene standards. In fact, the expectations should be focused on the reduction

of severe clinical mastitis cases (Bradley et al. 2015) or a long-term reduction of the basis reproduction rate of staphylococci on herd level (Schukken et al. 2014).

Udder Health & Milk Quality

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Evaluation of Two Drying Off Methods in Dairy Goats at the end of lactation.

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Objectives: To demonstrate the effectiveness of two dry off methods in goats: Group G1 was given an antibiotic therapy and Group G2 an antibiotic therapy followed by bismuth subnitrate at goat drying to reduce the number of animals affected with mastitis.

Materials and Methods: This study was carried out in dairy goat farms, located in the highlands of Mexico. Fifty-four dairy goats (108 glands) were sampled from following goat breeds: Saanen, Toggenburg and French Alpine, producing an average of 500 ml of milk per day, before dry. The animals were classified in two randomized groups, G1 (control group) of 29 goats (58 glands), and group G2 of 25 goats (50 glands). All goats were clinically examined, paying particular attention to udder health. Prior to milking, milk samples were collected from each gland and somatic cell count was determined through CMT, and at the same time with flow cytometry. The drugs used were selected based on in vitro antibiotic susceptibility results in milk for each gland. The dry off period consisted on an abrupt cessation of milking, accompanied by diet change and accommodation area. About two days prior to goat parturition, a cap of bismuth subnitrate was removed. Five days after parturition, milk samples were obtained for bacterial sensitivity and SCC were performed at this time. The percentage difference between negative and positive group mastitis in both cases, drying off and parturition, was performed using the following formula: $\% = ((CF / CI) - 1) \times 100$. The analysis of the corresponding proportions between the steps of drying off and parturition in each treatment was performed through bilateral contrast chi-square, X^2 statistical significance level of $\alpha = 0.05$.

Results: One hundred and eight goat glands (G1 and G2) were tested at dry off period; among them, 35 glands resulted mastitis negative, and at parturition, 91 glands represented an increase of 184.4% negative cases. At dry off period, 73 glands were positive for subclinical mastitis and at parturition, 16 glands resulted mastitis positive, which represent a 78% decrease. Milk samples collected during the study in both groups showed *Staphylococcus aureus* and *Streptococcus uberis*. Group G1 (only antibiotic therapy) showed 8.62% for positive cultures at dry off, and at parturition, 15.51%; in comparison with group G2 that showed 6% for positive cultures at dry off, and 2% at parturition. Analyzing subclinical mastitis, in group G1 a decrease of 70% was observed; versus in group G2 (Antibiotic treatment and sealant) that showed a decrease of 83%.

Conclusions: The antimicrobial therapy application followed by bismuth subnitrate provided a higher reduction in clinical mastitis versus just the administration of antibiotic therapy.

Bacteriological tests were conducted at the Laboratory of the Department of Medicine of Ruminants -FMVZ-UNAM

(Where: CF = final cases, CI = initial cases - 1 is the constant)

Udder Health & Milk Quality

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Efficacy And Economics Of A Polyvalent Mastitis Vaccine

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Objectives: Mastitis is an important animal disease causing decreased production and profits on dairy farms. J5 vaccines against gram negative pathogens have been used in the US for over 40 years. Since 2009 a polyvalent vaccine protecting against coliforms, *Staphylococcus aureus* and coagulase negative staphylococci (CNS) has been available in Europe (Startvac®/Topvac®). The objective of this study was to look at the efficacy of this vaccine under field conditions and to estimate the economic consequences of using this vaccine on dairy farms.

Materials and Methods: This abstract is based on the bachelor thesis written by Oostenbrug and Van Aaken in 2013 at Utrecht University, Faculty of Veterinary Medicine. To evaluate the efficacy of this vaccine a literature study with peer reviewed articles was performed. The data extracted from these papers is shown in the results section. For the economic analysis peer reviewed literature as well as case studies were used. The data extracted from these studies were then extrapolated to the Dutch situation in order to calculate costs of mastitis and possible costs and benefits of vaccination.

Results: Vaccine efficacy for coliforms for the first 120 days in milk (DIM) was evaluated in a UK study with over 3000 cows. No difference in incidence of clinical and subclinical mastitis was observed, but the severity of clinical mastitis cases was significantly reduced in the vaccinated groups. Furthermore vaccinated cows produced significantly more milk with higher milk solids than negative controls.

In Italy two herds with a total of 809 cows were vaccinated according to the label regime or left as controls. Vaccinated cows had a lower incidence of new infections with *S. aureus* or CNS and the duration of infection by *S. aureus* or CNS was significantly lower than negative controls. The estimated reproduction ratio (R_0) for *S. aureus* was decreased by 45% and for CNS with 35% compared to negative controls. Estimated vaccine efficacy decreased with increasing lactation number and significant differences between farms were observed.

As a result of the calculation and assumptions made in 2014 in regard to this vaccine, it can only give an annual cost of €40 per cow per year on a farm to be profitable.

In a UK study, vaccination with this vaccine resulted in a return on investment of 2.57:1 and gave an increase in milk production of 231L in the first 120 DIM. Based on the fact that milk production will most probably be the same this is an opportunity to extrapolate these figures for the Dutch situation. The culling rate was reduced with 8% when vaccination was performed. Using the same calculation method as mentioned before, vaccination may only cost €32,- or less to be profitable.

Conclusions: This vaccine has proven being capable of reducing clinical signs of acute coliform mastitis and reducing the reproduction ratio for more chronic mastitis pathogens. Also increased milk production with higher milk solids and reduced culling rates due to mastitis have been observed. Reductions vary between farms, indicating that a mastitis vaccine is not the sole solution for mastitis.

Based on two different studies, vaccination may only cost €32,- or €40,- or less to be profitable. It should be stated that the €32,- estimation is based on the first 120 DIM, indicating that this could be an underestimate for the costs of vaccination.

Udder Health & Milk Quality

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In practice case study to investigate the effect of selective dry cow treatment on mastitis incidence

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Objectives: In this case study a retrospective analysis was performed to investigate the effect of selective dry cow treatment on mastitis incidence measured by mastitis injectors sold in practice. Also the effect on several udder health parameters was analyzed.

Materials and Methods: For the retrospective analysis three periods were compared. The three periods are September 2012 to Sept 2013 (period 1), Sept 2013 to Sept 2014 (period 2) and Sept 2014 to Sept 2015 (period 3). For each period the following parameters were calculated on farms using selective dry cow treatment:

1. Total number of dry cow antibiotic injectors and internal teat sealers sold
2. Total number of mastitis injectors sold
3. Average Bulk milk somatic cell count in September for farmers (n=31) participating in specific management program (Pir Dap)
4. Average cow somatic cell count of culled cows in the management program (Pir Dap)
5. Culling percentage of farms in the management program (Pir Dap)

Selective dry cow treatment means only antibiotic dry cow treatment of cows with a cell count over 50.000 and heifers over 150.000 cells/ml.

The results of the last three parameters are compared to the data of farms not using selective dry cow treatment in the same period. Farms not using selective dry cow treatment are using dry cow antibiotics on every cow entering the dry period.

The first two parameters are available from the practice management system. The last three parameters from farms participating in a specific farm management program, called Pir Dap in The Netherlands. This data is also available for veterinarians with permission from the farmer.

Results: During the 3 year period a gradual decrease up to 10% in the number of dry cow antibiotic injectors sold was observed. Also a decrease of 14% was seen in the number of mastitis injectors sold. An increase of 27% in the number of teat sealers was noticed, but was less obvious in period 3. The evaluation of the bulk milk somatic cell count of 31 farmers participating in the Pir Dap management program showed an increase on 12 farms (39%), a decrease on 12 farms (39%) and on 7 farms (22%) the BMSCC remained stable. Half of the farms showed an increase of the average cow somatic cell count of culled cows and the other half a decrease. The culling percentage of 21 farms was analyzed and was on 11 farms reduced, increased on 7 farms and remained the same on 3 farms during the 3 year period.

The analysis of farms not using selective dry cow treatment (n=6) showed that BMSCC showed the same trend as farms using selective dry cow treatment (2 increased, 2 decreased, 2 remained the same), less farms had an increased cow SCC of culled cows (17% versus 48%) and more farms had a reduced culling percentage (67% versus 52%), compared to the farms using selective dry cow treatment. Additional evaluation of udder health parameters on farms using selective dry cow treatment will be performed.

Conclusions: By the number of dry cow and mastitis injectors sold we could conclude that selective dry cow treatment does not have a negative impact on mastitis. It is difficult to draw conclusions from the culling percentage since 2 factors played an import role. Due to the end

of the quorum system in 2014 less cows were culled. And in 2015 more cows were sold due to the low milk price.

We can conclude that farmers not using selective dry cow treatment seem to have a lower cow SCC at culling and a reduced culling percentage.

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A Diagnostic Tool for the Detection and Quantification of Pathogenic Bacteria in bovine milk samples from dairy farms in Bangladesh

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Objectives: The aims of the present study were: 1) to show the results obtained from milk samples from Bangladesh dairy farms, using an FTA card and PCR diagnostic tool (STARTCHECK®) provided by HIPRA and 2) to show that STARTCHECK® can be used to complement Somatic Cell Count (SCC) and Bacterial culture to monitor mastitis at a herd level by detecting the major mastitis causative agents (Staphylococcus aureus, Escherichia coli, Coagulase Negative Staphylococci (CNS) and coliform bacteria).

Materials and Methods: A total of 102 Bulk Tank Milk (BTM) samples and Pooled Mastitis Milk (PMM) samples were collected from different dairy farms in Bangladesh, between July and August 2015. The samples were taken by professionals according to the STARTCHECK® instructions [Drawing up 250ul of milk in a sterile manner (gloved hands) from the Bulk Tank (after 5 minutes of stirring) and 250ul of milk from the pooled mastitis cows in separate pipettes, then impregnating the designated areas (BTM and Mastitis Pooled) on the FTA card with the respective milk samples]. The inoculated FTA cards were sent with the farm details (number of lactating cows, average milk production and most recent Bulk Tank somatic cell count [SCC] reading) to DIAGNOS (HIPRA) in Amer, Girona, Spain via courier services. The samples were processed and tested using the Real- Time Multiplex PCR assay, to detect the presence of genetic material for S aureus, E. coli, Coagulase Negative Staphylococci (CNS) and coliform bacteria. The results were determined as positive or negative based on the Cycle threshold (Ct) values, with Ct values below 37 being considered as positive. The positive results were reported in a semi- quantitative manner, indicating the amount of genetic material obtained from each bacteria in the samples.

Results: All samples (100%) were positive for at least one bacteria. The prevalence of each pathogen from the Bangladesh data is as follows: Staphylococcus aureus, Escherichia coli, Coagulase Negative Staphylococci (CNS) and coliform bacteria were detected in 33%, 93%, 99% and 35% of samples respectively. Of these 102 samples 51 were BTM samples and 51 were PMM samples. The prevalence of each pathogen in its respective sample type is as follows: Staph aureus (27% and 39%), E. coli (88% and 89%), CNS (98% and 100%) and coliform bacteria (94% and 98%).

Conclusions: Mastitis is a multi- aetiological condition and needs to be managed with a multifactorial approach (good management and vaccination). STARTCHECK® has been used worldwide since 2009 and milk samples from 47 different countries have been analysed. STARTCHECK® is a good diagnostic and surveillance tool to identify pathogenic bacteria present at a herd level using BTM and PMM samples. The high specificity and sensitivity circumvents limitations

experienced with bacteriology (transport or poor growth) and the human error with individual cow SCC cut off level of 200 000 cells/ ml.

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Udder Health & Milk Quality

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The resistance to antibiotics and production of enterotoxins in coagulase negative staphylococci isolated from clinical mastitis in dairy cows

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Objectives: In recent decades, especially coagulase-negative staphylococci (CoNS) were diagnosed as ruminant mastitis agents in many countries, with significant impact on the economy of milk production. The aim of the work was the determination of the resistance to antibiotics and finding capacity to produce enterotoxins in coagulase negative staphylococci isolated from clinical mastitis in dairy cattle breeding.

Materials and Methods: During the two-year experiment in holding of 120 Slovak spotted cattle in Eastern Slovakia was udders of dairy cows examined clinically and bacteriologically a total of six times and each time the milk sample examined by NK-test (CMT). For the diagnosis of staphylococci was used set STAPHYtest 24 and evaluated by program TNW, version 7.0 ProAuto (Erba Lachema, Brno, CR). All isolated pathogenic bacteria CoNS (n = 41) from clinical mastitis were in vitro tested on Mueller-Hinton agar by zone disc methods (EUCAST, 2014) on resistance to 14 antibiotics: Ampicilin (10 µg); Amoxycilin (25 µg); Cloxacilin (5 µg); Cefoxitin (30 µg); Erytromycin (10 µg); Linkomycin (15 µg); Neomycin (10 µg); Novobiocin (5 µg); Penicillin (10 U); Streptomycin (10 µg); Ampicilin/Sulbactam (10µg); Oxacilin (5 µg); Cephalotin (30 µg); Tetracyklin (10 µg) (OXOID Ltd. Basingstoke, Hants, UK). Bacteria were rated as resistant or sensitive according reference zone by manual from producer. Identified bacteria CoNS were examined also on presence of genes coding their enterotoxigenic activity (sea, seb, sec, sed, see) by PCR method according to (Becker et al. 1998). Every strain with confirmed gene was examined for production of enterotoxins by Ridascreen ® Set A, B, C, D, E (R-Biopharm AG, Darmstadt, Germany).

Results: From a total of 732 individual milk samples, 218 positive accounted for 29.8%. From clinical mastitis cases were 7.9% (58) determined as the positive. Up to 5.6% (41) in cases of clinical mastitis bacteria were caused by CoNS. It was identified in 7 species *S. epidermidis* (13), *S. warneri* (9), *S. chromogenes* (7), *S. xylosum* (5), *S. sciuri* (3), *S. lentus* and *S. cohnii* (2). The bacteria in total caused 70.7% of clinical cases, which require antibiotic treatment. Highest value of antibiotic resistance bacterium CoNS isolated from clinical mastitis were determined to penicillin (15/41) and novobiocin (13/41). The presence of genes coding production of enterotoxins in bacteria coagulase negative staphylococci (n = 41) was as follows - tree strains of *S. epidermidis* showed presence of gene sea (1), and sec (2), but by ELISA method was confirmed only production SE types A (1) and C (1) in two strains; in *S. chromogenes* and *S. warneri* was determined presence of one gene sec, but only in *S. chromogenes* was conjugated with production correspondent type of SEC; in *S. xylosum* was found gene sed. Was confirmed the presence of the gene (seg) encoding

staphylococcal enterotoxins (SE) in 4 (9.7%) cases, in vitro production of SE was observed in 3 (7.3%) cases.

Conclusions: Tested coagulase negative staphylococci from 41 clinical mastitis cases showed high resistance to penicillin, especially for bacteria *S. epidermidis* (5/13), *S. chromogenes* (3/7), *S. xylosum* (2/5) and novobiocin, especially for bacteria *S. epidermidis* (6/13), *S. warneri* (3/9), *S. chromogenes* (3/7). In bacteria *S. epidermidis* (3), *S. chromogenes* (1), *S. warneri* (1), *S. xylosum* (1) were by PCR detected the presence of genes sec (4), sea (1) and sed (1), but production of SE by ELISA method only in *S. epidermidis* SEA (1) and SEC (2) in *S. epidermidis* and *S. chromogenes*.

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Udder Health & Milk Quality

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RAPD analysis of genome diversity and detection of virulence-associated genes in Staphylococcus aureus isolated from bovine mastitis

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Objectives: *Staphylococcus aureus* is recognized as a pathogen causing many serious diseases in humans and animals and is one of the most common etiological agents of clinical and subclinical bovine mastitis. The purpose of this study was to investigate the genome diversity of *S. aureus* strains (n=46) isolated from bovine mastitis in Beijing, Hebei and Liaoning by RAPD (randomly amplified polymorphic DNA) and to determine the presence of genes encoding *fnbA*, *fnbB*, *hla*, *hly*, *pvl*, *etA*, *etB* and *tst* by PCR (polymerase chain reaction amplification).

Materials and Methods: A random primer: 5'-TGACCCGC-3' was used to amplify the genome DNA of *Staphylococcus aureus*, and then the amplification products were separated by gel electrophoresis and photographed under UV light. The RAPD profiles were compared on the basis of the presence or absence of each DNA band, and strain diversity was calculated using the Jaccard's coefficient and UPGMA (unweighted pairgroup arithmetic average clustering) cluster analysis. In addition, primers for the virulence-associated gene were designed using DNAMAN, respectively

Results: RAPD results showed that all the *S. aureus* isolates produced 2-7 different banding patterns and were divided into 10 genotypes at the identity level of 55%. A significant variation of genotypes was found in different areas like that genotype I and II in Beijing, genotype VIII and IX in Hebei and genotype III in Liaoning province. In addition, all isolates were found to harbor one or more virulence-associated genes, and the most frequently encountered genes were *hla*(100%), *hly*(100%) and *tst*(100%), followed by *fnbA*(70%), *fnbB*(33%), *etA*(24%), *pvl*(4%) and *etB*(2%), respectively.

Conclusions: The diversity of *S. aureus* genotypes in areas of Beijing, Hebei and Liaoning provinces indicated that environmental conditions may be an important factor. Furthermore, the relatively wide distribution of the genes *hla*, *hly* and *tst* suggested that they play a prominent role in bovine mastitis pathogenesis. The present study provides a valuable insight into the genotype and virulence associated genes of this important pathogen.

Comments: The present study provides a valuable insight into the

genotype and virulence associated genes of *Staphylococcus aureus* isolated from bovine mastitis. This research was supported by No.2012bad12b03, No.313054, No.20120008110042 and No.31572587

Udder Health & Milk Quality

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Sequence Analysis Of Coagulase Genes Of *Staphylococcus Aureus* Isolated From Mastitis

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Objectives: Mastitis is a major dairy herd problem mainly caused by *Staphylococcus aureus*. The present study was conducted to determine the relationship of coagulase gene of *Staphylococcus aureus* between Pakistani and other world strains.

Materials and Methods: The milk samples were collected from mastitic buffaloes. DNA extraction was performed as prescribed by Genomic DNA Purification Kit. DNA amplification was carried in thermocycler (Kyratec SC 200) with following cyclic conditions: Initial denaturation at 95 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at 52 °C for 30 sec for Coagulase gene. Then extension at 72 °C for 30 sec. A final extension at 72 °C was carried out for 5 min. All the PCR products were run on 1.2 % agarose gel stained with ethidium bromide and visualized under UV light in Gel Documentation System (BioRad USA). The isolated strains were subjected to sequence analysis of coa gene and phylogenetic tree (using MEGA6.1 software package) was constructed.

Results: It revealed that the coa gene of *S. aureus* strains, isolated from mastitic buffalo milk samples, could be grouped in two clades which were closely related to *S. aureus* isolates from Japan, India and Taiwan, while *S. aureus* isolates from Germany, UK and USA were distantly related. These results indicated the genetic relatedness of Pakistani isolates with other reported isolates from different parts of the world. All the Pakistani strains of coagulase gene were 99.98-100% similar while their divergence was 0.02%. The Japanizes strains were close to Pakistan strains and their similarity and divergence was 99.97% and 0.03%, respectively. The Pakistani strains were 99.93% similar and 0.07% divergent from Indian and German strains. Similarly, the Pakistani strains were 99.94% similar and 0.06% were divergent from UK and Taiwan reported strains.

Conclusions: This particular knowledge may support in effective diagnosis, treatment and control of mastitis in river buffaloes in Pakistan. These findings will also be helpful in future for designing suitable mastitis control strategies in the country.

Udder Health & Milk Quality

P04-004-210

A Diagnostic Tool For The Detection Of Bacteria (*Staph Aureus*, *E.Coli*, Cns And Coliforms) In Bovine Milk Samples From Dairy Farms In South Africa

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Objectives: The aims of the present study were: 1) to show the results obtained from milk samples from South African dairy farms over the past three years, using an FTA card and PCR diagnostic tool (STARTCHECK®) provided by HIPRA and 2) to show that STARTCHECK® can be used to complement Somatic Cell Count (SCC) and Bacterial culture to monitor mastitis at a herd level by detecting the major mastitis causative agents [*Staphylococcus aureus*, *Escherichia coli*, Coagulase Negative *Staphylococci* (CNS) and coliforms].

Materials and Methods: A total of 301 samples [151 Bulk Tank Milk (BTM) samples and 150 Pooled Mastitis Milk (PMM) samples] were collected from different dairy farms in South Africa, between January 2012 and February 2015. The samples were taken by veterinarians according to the STARTCHECK® instructions [Drawing up 250ul of milk in a sterile manner (gloved hands) from the Bulk Tank (after 5 minutes of stirring) and 250ul of milk from the pooled mastitis cows in separate pipettes, then impregnating the designated areas (BTM and PMM) on the FTA card with the respective milk samples.]. The inoculated FTA cards were sent with the farm details (number of lactating cows, average milk production and most recent Bulk Tank SCC reading) to DIAGNOS (HIPRA) in Amer, Girona, Spain via ordinary post or courier services. The samples were processed and tested using the Real-Time Multiplex PCR assay, to detect the presence of genetic material for *Staph. aureus*, *E. coli*, CNS and Coliform bacteria. The results were determined as positive or negative based on the Cycle threshold (Ct) values, with Ct values below 37 being considered as positive. The positive results are semi-quantitative indicating the amount of genetic material obtained from the samples correlating to the prevalence of the pathogen in the herd taking into account dilution factors.

Results: A total of 266 samples (96,3%) were positive for at least one bacteria (*Staph aureus*, *E. coli*, CNS and Coliforms), while 3,7% of the samples tested were negative for all four pathogens. Of these 266 samples, 144 were BTM samples and 166 were PMM samples. The prevalence of each pathogen in its respective sample type (BTM and PMM) is as follows: *Staph aureus* (19,4% and 32%), *E. coli* (59,7% and 73,7%), CNS (96,5% and 94,3%) and Coliforms (30,5% and 44,2%). The prevalence of each pathogen from the South African data is as follows: *Staph aureus* 26%, *E. coli* 66%, CNS 96% and Coliforms 38%.

Conclusions: Mastitis is a multi-aetiological condition that needs to be managed with a multifactorial approach. STARTCHECK® has been used worldwide since 2009 analysing samples from 44 different countries. STARTCHECK® is a good diagnostic/surveillance tool that can identify bacteria at a herd level using BTM samples. The high specificity and sensitivity circumvents limitations seen with bacteriology and SCC cut off levels of 200 000 cells/ml. STARTCHECK® has semi-quantitative properties. Compared with worldwide data, the presence of *Staph. aureus* in South African samples (26%) is higher than samples obtained from other countries (6-10%).

Udder Health & Milk Quality

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A Controlled Field Study Investigating The Effects Of A Mastitis Vaccine In A *Staphylococcus Aureus* Positive Dairy Herd

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Objectives: Despite improvements in management, *S. aureus* remains a major cause of mastitis. Its chronic and destructive nature often leads to premature culling and ample loss of revenue. Additional help was sought in the form of a mastitis vaccine to overcome this challenge. The study investigated the ability of a mastitis vaccine to reduce new intramammary infections (IMI) with *Staphylococcus aureus* (*S. aureus*), improve bacterial cure, and reduce the duration of IMI and to investigate its effect on milk somatic cell count.

Materials and Methods: A total of 164 multi- and primiparous cows from a herd with 251 lactating cows with a 16.51% herd prevalence of *S. aureus* were enrolled into this controlled study. The selection of the treatment and control cow groups were based on the principle of matching. Cows were matched according to their parity, milk yield, *S. aureus* intramammary infections and the number of quarters infected per udder, the level of somatic cell counts and the udder parenchyma status based on results from udder palpation. Half of the remaining lactating cows in the herd not selected were also vaccinated to allow for an even exposure of vaccinated and control cows in an attempt to limit the effect of partial protection or exposure of herd mates. Selected cows were vaccinated on days 0, 35 and 87 of the study and quarter milk samples were collected at day 0 and monthly thereafter for 5 consecutive months for micro-cytology analysis. The Startvac (Hipra Southern Africa) mastitis vaccine was used. It contains an inactivated *S. aureus* (CPS) SP140 strain expressing Slime Associated Antigenic Complex (SAAC) and an inactivated *Escherichia coli* (J5). The generalized linear mixed model (GLMM) was used to evaluate somatic cell counts and milk yield over time was analysed using a linear mixed model for repeated measurements. Mean values were separated using Fisher's protected least significant difference test (FPLSD) and the Studentized range at the 5% level.

Results: This study demonstrated a significant ($P < 0.001$) increase of new *S. aureus* IMI in control cows while numerically fewer quarters were newly infected in the vaccine groups (0.88% to 2.56%). The duration of the quarter infections in the vaccine groups was 26.3% shorter (4.3 to 3.6 samplings months) than those in the control group. The combination of the reduced duration and fewer new infections in vaccinated animals contributed in reducing the overall exposure of cows within a herd to *S. aureus* IMI. This was evident in the significantly ($P < 0.05$) higher numbers of *S. aureus* isolated from the control group during the study.

The number of *S. aureus* clinical flare ups present in the vaccine group was significantly ($P < 0.05$) less and their cure rate higher although not significantly (44.23% compared to 34.55% in the control group). Significant ($P < 0.001$) differences in the SCC were found between sample periods but not between groups. Somatic cell count was 9.68% lower in the vaccine group regardless of udder infection status. A non-significant daily average gain in milk yield of 0.73 kg was evident in the vaccine group for the six months study period.

Conclusions: We found the overall effect of the vaccine to be moderate with significant and often marginal improvement evident in the vaccine group. Improved cure rate alongside a shorter duration of intramammary infections lower the transmission rate of the bacteria in the herd and indirectly reduced the overall exposure of individuals to *S. aureus*. Less udder parenchyma damage may lead to a perceived increased longevity of animals. To effectively exploit the advances acquired from use of the vaccine and to lower the reproductive ratio of *S. aureus* in a herd, the vaccine should always be used in combination with excellent parlour management and hygiene.

Udder Health & Milk Quality

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A model approach to estimate *Staphylococcus aureus* intramammary infection dynamics for different scenarios when vaccinating for mastitis

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Objectives: When new remedies or treatments are considered for used in a farming enterprise the main concern is whether the benefit will outweigh the cost. Benefits however should not only be measured in economic terms. In addition to stochastic partial budget models that estimate cost and benefits when using Startvac (Hipra) mastitis vaccine, a model was developed to calculate the intramammary infection (IMI) dynamics of *Staphylococcus aureus* (*S. aureus*) at end points. The transmission rate of *S. aureus* depends on various factors, but parlour management and pathogenicity of bacteria are prominent.

Materials and Methods: The reasoning that subclinical *S. aureus* IMI either advanced to clinical mastitis, cure spontaneously or persist was exploited. It was anticipated that the percentage of persistence, cure and clinical cases would differ between old and new *S. aureus* IMI. The transmission parameter (β) used in low and high risk scenarios and varied from 0.0028 to 0.046 new infections daily. The duration of a persistent *S. aureus* IMI was taken as 115 days when few interventions took place. This duration was shown to decrease to 51 days in low somatic cell count herds, when an aggressive approach to detect and to treat subclinical *S. aureus* cases were in place. According to research the basic reproduction ratio (R_0) of *S. aureus* IMI was reduced by approximately 45% with the use of Startvac mastitis vaccine in a herd.

Four different scenarios, all with a *S. aureus* IMI herd prevalence of 15% were explored. The different β for *S. aureus* were combined with and without treatment of subclinical *S. aureus* cases. In all four scenarios vaccinated and nonvaccinated herds were compared.

The model explored two scenarios in herds with a low transmission risk: {scenario A} with an estimated persistent *S. aureus* infection of 115 days {scenario B} and an estimated persistent *S. aureus* infection of 51 days. In addition IMI dynamics were calculated in herds with a high transmission risk: {scenario C} without treatment of subclinical cases and an estimated persistent *S. aureus* IMI across cows of 115 days and {scenario D} with aggressive treatment and an estimated persistent *S. aureus* infection of 51 days.

Results: In scenario A it was estimated at day 153, that 3.37 clinical cases had occurred and 0.01 *S. aureus* cases persisted in the non-vaccinated group compared to 3.13 clinical cases no persistent cases in the vaccinated group. In a non-vaccinated herd during scenario C after 51 and 204 days the persistent *S. aureus* cases increased from 17.02 to 28.19 and clinical cases from 7.50 to 39.15 compared to a reduction in persistent cases from 9.36 to 1.42 and a smaller rise in clinical cases from 5.4 to 13.04 cases when vaccinated. The differences in persistence and clinical cases between non- and vaccinated groups in Scenario D were estimated at day 255 to be 54.60 and 81.20 compared to 16.52 and 30.96 respectively. Results indicated that the longer this mastitis vaccine was used the more distinct its benefits became. During a low risk scenario when vaccination was used in combination with treatment of subclinical *S. aureus* cases, *S. aureus* IMI was eradicated an estimated 51 days earlier than when treatment was used by itself. More pronounced effects were also detected in all high risk scenarios. Persistent *S. aureus* IMI continue to increase in high risk scenarios where subclinical *S. aureus*

cases were only treated but decreased under the same conditions when treatment was combined with vaccination.

Conclusions: The model revealed that the use of this mastitis vaccine provided additional benefits to combat *S. aureus* IMI in both high and low transmission risk scenarios. Less clinical cases occurred from the first cycles and intensified with duration. The only high transmission risk scenario, where persistent and clinical cases were reduced over time was when treatment of subclinical cases was used in combination with the mastitis vaccination.

Models such as this have practical applications and can assist both veterinarians and producers in optimizing predicted cost, benefits and can including the IMI dynamics of *S. aureus* into in their future planning.

Udder Health & Milk Quality

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An evaluation of the efficacy of *Staphylococcus aureus* vaccination for the management of mastitis in New Zealand dairy cows

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Objectives: The objective of this study was to investigate the role of Startvac® vaccination for the management of mastitis in New Zealand dairy cows under field conditions. The study enrolled 15 farms across 3 main dairy regions in New Zealand.

The demonstration of vaccine efficacy in a clinical field trial can be challenging as vaccination may affect the basic reproductive number (R_0) for the infectious organism within the herd. This study was designed at the herd level to mitigate that effect.

Materials and Methods: A total of 15 farms across 3 main dairy regions in New Zealand were enrolled into the study. This consisted of 902 cows from 3 farms in Taranaki, 2427 cows from 4 farms in Canterbury, and 5451 cows from 8 farms in Southland. A total of 3581 cows from 7 farms were vaccinated with Startvac®, 2302 animals from 4 farms were vaccinated with a placebo solution (sterile saline), and 2897 animals from 4 farms were left unvaccinated.

Individual somatic cell counts (ISCC) reflecting intra-mammary infections (IMI) were used to assess the longitudinal effect of Startvac® vaccination within herds. The differences in daily herd somatic cell counts (BMSSC), somatic cell count linear scores (SCCLS), and the proportion of animals with clinical mastitis (CM) were calculated between seasons and compared between treatment groups.

A cut off value of 200,000 cells/ml was used to calculate the proportion of cows with mastitis, and the proportion of uninfected, new, recovered, chronic and repeat infections between two subsequent herd tests. For Southland farms, milk samples were collected by the farmers and cultured to determine the bacteriological status.

Herd test data, bulk tank somatic cell counts and mastitis data were obtained electronically using Infovot and MINDA. MS Excel, MS Access and R were used for database management and data analysis. The treatment effects were estimated by comparing the means of the between season differences (pre and post vaccination) for each farm and treatment group.

Results: The proportion of intramammary infections (ISCC >200,000 cells/ml) at each herd test ranged from 9.3% to 31.0% for 2013-2014 and 9.8% to 26.8% for 2014-2015. The herd level between-season differences in IMI proportion ranged from -21.1% to 6.4%.

A between season reduction in intramammary infections of 3.2% was observed in vaccinated group, whereas the control and placebo control herds experienced an increase of 0.8% and 1.3%, respectively.

The proportion of uninfected animals increased in the vaccinated group (4.8%) and decreased in the control and placebo control farms (-2.5%; -0.7%).

The proportion of new infections reduced in vaccinated farms (-2.1%), reduced slightly in placebo farms (-0.4%) and increased in control farms (1.5%).

The proportion of chronic infections reduced in vaccinated farms (-2.2%) but increased in control and placebo farms (0.4%; 1.6%).

The proportion of repeat infections decreased in all farms (-0.2%, -1.0% and -0.8% for vaccinated, control and placebo respectively).

When compared with the control group, vaccinated herds experienced an average between season reduction of 29.53 (000's) cell/mL, and the placebo control group experienced an increase of 35.16 (000's) cells/mL.

A mastitis incidence risk of 10.9% was calculated for vaccinated herds, whereas control and placebo control herds averaged 8.6% and 8.9% respectively. The increase observed in the vaccinated group was attributed to a single farm experiencing a spring *Streptococcus uberis* outbreak.

Conclusions: The results of this study suggest that StartVac® vaccination may have a positive effect on udder health and could be a useful tool for the management of mastitis under New Zealand field conditions. Furthermore, the results of this study suggest that the placebo effect of trial participation may have an effect on the outcome. Future large scale studies will be beneficial for our understanding of both the herd level response to vaccination, and to better understand the placebo effect of trial participation.

Udder Health & Milk Quality

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Colonization of *Staphylococcus aureus* in the mucosal inflammatory area in the case of severe clinical bovine mastitis

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Objectives: *Staphylococcus aureus* causes intractable bovine mastitis and is the one of the most significant mastitis-related problems for dairy farms. Colonization of *S. aureus* in mammary epithelial cells or cell lines, which stem from the lobule with alveoli (LA), has been detected via in vitro situations. Because limited information is available regarding local colonization of *S. aureus* in intramammary infection in udders with bovine mastitis, we established a clinical mastitis model in cows by experimentally infecting with *S.*

aureus to detect the colonization of *S. aureus* via in vivo observation in mammary tissues.

Materials and Methods: To detect colonies of *S. aureus* in the udder, we established 5 bovine mastitis models using experimental infection with *S. aureus*. Clinical symptoms were recorded twice a day, at the morning and evening milking, for 1 week. Clinical scoring criteria were used to determine the severity of clinical mastitis, utilizing basically the same format previously described (2009, *Vet Microbiol.* Atalla, et al). Milk was bacteriologically analyzed, and the somatic cell counts (SCC) of the milk was determined. Tissue samples collected from the LA, gland cistern (GC), and teat cistern (TC) were fixed in 10% phosphate-buffered formalin and embedded in paraffin for histochemical analysis. In addition, to clarify whether hematoxylin aggregation represented *S. aureus* colonies, we compared hematoxylin aggregation, gram-positive cells, and *S. aureus* positive cells stained with immunofluorescence using serial sections.

Results: During the 1-week observation period, the milk from all of the udders infused with *S. aureus* had increased SCC and numbers of *S. aureus*; these cows also exhibited clinical symptoms. Based on a scoring system for mastitis severity, 2 cows were classified with moderate mastitis, and 3 cows were classified with severe mastitis. The histochemical analysis showed that a mucosal inflammatory reaction (i.e., broken epithelium and dampness of neutrophils) was present in the entire udder, including not only the LA but also the GC and TC. In addition, aggregation of hematoxylin, gram-positive cells, and *S. aureus* positive cells was localized in the same areas on the mucosal inflammatory areas in severe clinical mastitis. Therefore, with intractable mastitis caused by *S. aureus*, the bacteria could potentially easily colonize various locations of the udder such as the LA, GC, and TC.

Conclusions: Our results demonstrate that mastitis caused by *S. aureus* was reflected in mucosal inflammation of all of the mammary tissue including the LA, GC, and TC, at least in udders infected with *S. aureus*. Furthermore, *S. aureus* colonized near the broken mucosal barrier in severe clinical mastitis. These findings provide information for the mechanism, prevention, diagnosis, and therapy of mastitis caused by *S. aureus* infection.

Udder Health & Milk Quality

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DETERMINATION OF THE MILK QUALITY BOVINE PRODUCED IN THE MUNICIPALITY OF TARIMBARO MICHOACAN

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Objectives: The aim of this study was to determine the milk quality of bovine produced in the municipality of Tarimbaro Michoacan, through California test, Lactoscan and Microbiological Control.

Determine the percentage of butterfat, protein, and colony forming units/ml in milk samples obtained Tarimbaro municipality, Michoacan.

Materials and Methods: The study was conducted from september of 2012 to may of 2013 in the municipality of Tarimbaro, Michoacan. 100 dairy herds of the same number of production units of municipality

Tarimbaro, Michoacan during the morning milking were sampled. To do this, a milk sample pot of receipt of each herd, previously mixed with a tablespoon placing the sample in a plastic pallet for California test was taken. Then the other part of the sample is poured into a glass jar previously sterilized. Subsequently, we proceeded to place samples in a sealed cooler at a temperature of 4 °C for transport immediately bacteriology laboratory of the Faculty of Veterinary Medicine University Michoacana of San Nicholas of Hidalgo for processing. Samples were analyzed by casting technique plate, both for coliform and for mesophilic aerobic bacteria (BMA).

Results: It was found that 94% of the samples were negative, and 6% grade traces with CMT, that is of excellent quality according to international standards. Regarding the determination of butterfat in gL with Lactoscan analyzer based on the NM-F700-COFOCALEC 2004 standard, it was found that 69% of the samples corresponded to class A, 5% to class B, 6% to class C, while the remaining milk (20%) was found outside the norm. As for the results of bacteriological analysis for BMA, these are also interpreted according to the NM COFOCALEC F-700, (2004), found that 46% of the samples corresponded to class 1, 100,000 CFU/ml, 11% to Class 2, 12% in Class 3, 7% to the Class 4, while the rest (24%) containing It was found that 94% of the samples were negative, and 6% grade traces with test of California, that is of excellent quality according to international standards. Regarding the determination of butterfat in gL with Lactoscan analyzer based on the NM-F700-COFOCALEC 2004 standard, it was found that 69% of the samples corresponded to class A, 5% to class B, 6% to class C, while the remaining milk (20%) was found outside the norm. As for the results of bacteriological analysis for BMA, these are also interpreted according to the NM COFOCALEC F-700, (2004), found that 46% of the samples corresponded to class 1, 100,000 CFU/ml, 11% to Class 2, 12% in Class 3, 7% to the Class 4, while the rest (24%) containing more than 1,200,000 CFU/ml, were classified outside the provisions of this standard. more than 1,200,000 CFU/ml, were classified outside the provisions of this standard.

Conclusions: It is concluded that the milk produced in the municipality of Tarimbaro, Michoacan is good quality, and to comply with the parameters established nationally and internationally. Making it suitable for consumption by the public consumer without risk.

Udder Health & Milk Quality

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Best practice approach of Streptococcus agalactiae infection on a dairy farm

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Objectives: Our objective was to set up a best practice approach of Streptococcus agalactiae (SAG) infection on a dairy farm. SAG is known to be very contagious on a dairy farm and can cause high cow somatic cell counts (SCC). Often a whole or part herd treatment is applied when SAG infection on a farm is diagnosed. In this case-study, diagnostic testing and treatment decisions on the individual cow level was used as a strategy in order to avoid spread of the pathogens and to use as less antibiotics as possible.

Materials and Methods: On the farm were this strategy was performed, the dairy cows were housed in a free stall barn build in 2011 with a capacity of 450 cows, inclusive dry cows. The barn had closed floors, straw with lime and water added was used as bedding material. The

cows were milked three times daily. At drying off all cows were treated with a small spectrum dry cow antibiotic injector containing cloxacillin and a teat sealer. Although *Staphylococcus aureus* (SAU) was the major pathogen present in the bulk milk samples on this farm, additional SAG infection on the farm was suspected after isolation of the pathogen in a bulk milk sample. At the time SAG was diagnosed the bulk milk somatic cell count (BMSCC) was 293.000 cells/ml.

Initially only cows with new elevated (> 250.000) SCC were sampled for bacteriological culture, but no SAG was detected. Even though the bulk milk showed SAG again. From all cows (n=325) a composite milk sample was taken.

Results: Most dominant pathogens found were Coagulase Negative Staphylococci (CNS)(n= 36), penicillin resistant SAU (n=28) and penicillin sensitive SAU (n=21). In only 3 cows SAG was found. One SAG cow was culled because it had a mixed infection with SAU and a history of high SCC. The other 2 SAG cows were treated with a second choice intramammary injector and systemically with trimetoprim-sulfa, both cows cured. Besides that a separate milking group with high SCC cows was formed. All the cows with penicillin resistant SAU, penicillin sensitive SAU, CNS and *Serratia* species were housed in this group. This group was milked at the end of milking and cows could not leave this group only when culled or after reduced somatic cell count and a negative culture. Individual treatment decisions for this group were based on SCC history, SCC elevation, days in milk and age. The measurements resulted in disappearance of SAG from the bulk milk. Nevertheless the bulk milk somatic cell count did not decrease. Some SAU infected cows in the separate milking group should have been culled, but due to lack of replacement animals those cows remained in the herd. This is also the reason why the number of new infections dropped at first (4%), but increased again after a few months (11%). Twenty to thirty percent of those "new" infections are cows with still a high SCC after the dry cow period (not cured).

Conclusions: Since only 3 cows were found SAG positive we could wonder if SAG is really highly contagious. This particular SAG could have been a more human like and less contagious strain. In this case elevated cow SCC was not a valid criteria for diagnosing SAG infection in cows.

This practice approach led to the treatment of cows that had a good chance to cure only. Therefore minimal antibiotics were needed to tackle the SAG infection in this herd, compared to a more common approach for whole or part herd treatment. Perhaps a more sustainable effect on bulk milk somatic cell count could have been reached if more high SCC and infected cows were culled.

Udder Health & Milk Quality

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Biochemical, Microbiological and Cellular Profiles of Crossbred Cow's Milk at Delivery to 180 Days of Lactation

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Objectives: This study evaluated the incidence of pathogens, such as Somatic Cells Count, Total Bacterial Count, biochemical evaluation and

differential cell of 225 milk samples from February 2012 to November 2012.

Materials and Methods: Twenty-five crossbred cows, pluriparous, from a commercial farm located in the district of Ibitira, Martinho Campos, Minas Gerais were used. To collect, pre-dipping routinely made by the farm, then cleaning the ceilings with gauze soaked in 70% alcohol was performed. After this procedure the disposal of the first three streams of milk was performed and conducted the store. The collections were made on the exact day of delivery and colostrum, 5, 10, 30, 60, 90, 120, 150 and 180 days thereafter. After milk collection, the slides were prepared and stained for Total Bacterial Count, estimated somatic cell count and differential cell count. The remaining material was used for bacterial isolation and biochemical evaluation.

Results: In somatic cell count, decreased between the third and fourth months of lactation and increased for the middle third lactation was observed. About 48% of the animals evaluated at all times had Somatic Cells Count above 200,000 cells/ml of milk. For the presence of pathogens, of 25 cows, 18 (72%) were positive for *Staphylococcus aureus* in early lactation and only this pathogen was present until the end of the experiment. Cows that have high somatic cell counts also show increased concentration of magnesium chlorides and calcium in the whey. According to the cellularity of milk, this work presented as predominant in milk cells neutrophils, followed by lymphocytes and macrophages and the concentration of chlorides, magnesium, calcium, alkaline phosphatase and GGT in milk, accompanied increasing the concentration of lymphocytes in the same.

Conclusions: The most prevalent pathogens in the course of this study were *Staphylococcus aureus*, *Streptococcus uberis* and *Bacillus* spp. In biochemical evaluation in automatic device, when there was an increased concentration of somatic cells, there was an increase in the concentration of chloride, calcium, magnesium, alkaline phosphatase and gamma- glutamyl transferase in milk. The differential cell performed by optical microscopy, was increased when the concentration of somatic cells, it increased the concentration of neutrophils, lymphocytes, eosinophils, macrophages, epithelial cells and basophils.

Udder Health & Milk Quality

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An investigation on the efficacy of a vaccine against *Staphylococcus aureus* under field conditions in Northern Italy

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Objectives: The aim of this study was to evaluate the efficacy of a commercial vaccine (Startvac®, Hipra Spain) aimed at reducing intramammary infections (IMI) by *Staphylococcus aureus* and coagulase-negative staphylococci under field conditions.

Materials and Methods: This study was carried out in a farm in Northern Italy, which was selected basing on the size of the herd (450 lactating cows). Cows were randomly allocated in two groups. The first group received the vaccine following the label regime, and the second group was left unvaccinated to act as controls. The two groups were housed in the same pen. Four aspects of vaccine effectiveness were

considered: the first includes the impact of vaccination on the rate of new infections, second it concerns the impact of vaccination on the reduction of shedding of *S. aureus* from an infected animal, third it addresses the impact of vaccination on the rate of cure of the infection; and the finally, the fourth aspect concerns the reduction of clinical symptoms related to infection due to vaccine effectiveness. Vaccination of animals was made according to the registration protocol of the vaccine for a total of three doses, with the first dose at 45 days before the estimated date of calving, the second administration at 10 days before the date of calving, and the third dose at 52 days post-calving.

Results: Results refers to about half of the trial duration (18 months). The prevalence of *S. aureus* in the studied herds was about 3% at single quarter level for vaccinated cows, while the prevalence of coagulase-negative staphylococci amounted to approximately 6%. The incidence of new staphylococcal IMI was lower in the vaccinated group than in the control group. Results showed a moderate difference in *S. aureus* prevalence in milk samples examined between vaccinated and control animals (3% in vaccinated group; 4% in control group). However, the average duration of infection by *S. aureus* was significantly shorter in vaccinated animals compared to control animals. We detected also a lower number of cases of clinical or subclinical mastitis in the vaccinated group than in the control group.

Conclusions: The results indicate that the vaccine can be an effective tool to control staphylococcal mastitis, by reducing the incidence and duration of the infection. As reported in other studies, mastitis vaccination can play an important role in the mastitis control programs (Schukken et al, 2014; Bradley et al, 2015).

Udder Health & Milk Quality

P04-004-219

Elimination of bacteria in experimentally induced *S. epidermidis* intramammary infection assessed by PCR and plate counting

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Objectives: The aim of this study was to follow-up infection and inflammation process in experimental *Staphylococcus epidermidis* intramammary infection (IMI) using milk somatic cell count (SCC) and two bacteriological methods, conventional bacteriological culture (BC) and real-time PCR. Our hypothesis was that IMI would be eliminated faster when assessed with BC than assessed with PCR, as PCR amplifies also DNA of dead or damaged bacteria.

Materials and Methods: One quarter free from bacterial growth and with milk SCC<100,000 of eight primiparous cows was experimentally infected with *S. epidermidis* (PM221). The inoculum contained 5.7 × 10⁶cfu in 7 ml of saline. Aseptic milk samples were collected at -24h and 0h before the challenge and then at 4, 6, 12, 21, 27, 30, 36, 45, 54, 69, 78, 93, 102,117, 126, 141, 168, 240 and 336h post-challenge (PC). 100 µl and serial dilutions of each sample were cultured on blood agar for bacterial counting. Detection limit for a positive result was 10 cfu/ml of milk. Colonies were identified as CNS by standard procedures. Samples were also tested with real-time PCR (PathoProofTM Mastitis Complete-12 Kit, Thermo Fisher Scientific Ltd, Finland). Cycle threshold (Ct) values were measured up to 40.0. Ct cut-off limit for a positive result was 37.0. Milk SCC was determined with Fossomatic (Valio Ltd, laboratories Finland).

Results: All quarters became infected with *S. epidermidis*. For all cows mean bacterial counts of the two-week period were 1.93 log₁₀ cfu/ml (SD 1.49), mean Ct value was 31.17 (SD 4.15) and SCC 2.865,174 cell/ml (SD 3.668,413). According to both diagnostic methods, the amount of *S. epidermidis* was the highest at the second sampling 6h PC, when bacterial count was 4.02 log₁₀ cfu/ ml (SE 0.38) and in PCR the mean Ct-value 27.74 (SE 1.46).

Based on BC, 3 out of 8 cows were spontaneously cured within the two week period and five cows remained persistently infected. Infections were eliminated i.e. no bacterial growth was detected after 54h (Cow 1), 240h (Cow 2) and 45h (Cow 6) PC. SCC at these time points were 8 988 000 cells/ml, 517 000 cells/ml and 4 063 000 cells/ml.

Assessed with PCR, only one cow was tested negative at time point 240 h PC (Ct value 40.0, Cow 1). At this point, milk SCC of the quarter was 57 000 cells/ml.

Conclusions: The amount of *S. epidermidis* bacteria was the highest at 6h PC assessed with both diagnostic methods. Within the two-week period, 3/8 quarters were cured according to BC results, but only one with PCR. Quarters defined cured with BC had still high milk SCC, which is not surprising as inflammation disappears slower than infection. Milk SCC of the quarter defined cured with PCR was low, indicating also resolved inflammation in that quarter. PCR may give positive results for longer than culture, as it amplifies also DNA from dead, damaged and growth-inhibited bacteria. Thus control samples after IMI may be advisable only after decreased SCC.

Udder Health & Milk Quality

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Bovine *Staphylococcus aureus*: subtyping, evolution and zoonotic transfer

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Objectives: *Staphylococcus (S.) aureus* is globally one of the most important pathogens causing mastitis in cattle. Former studies, however, demonstrated in Swiss cows that *S. aureus* isolated from bovine intramammary infection (IMI) is genetically heterogeneous. *S. aureus* genotype B (GTB) is contagious whereas all the remaining genotypes are involved in individual cow disease. The aim of the present study was to compare 3 subtyping methods using strains of *S. aureus* isolated from IMI and bulk tank obtained from various European countries. The phylogeny of animal *S. aureus* was inferred and the zoonotic transfer between cattle and humans was studied.

Materials and Methods: 456 bovine strains of *S. aureus* were examined that were obtained from 12 European countries. The isolates originated from aseptically collected milk samples from single-quarters (SQM) with subclinical IMI, from bulk tank milk (BTM) or from own strain collections (subclinical IMI). The strains were subtyped by ribosomal spacer PCR (RS-PCR; Fournier et al., 2008), spa typing (Harmsen et al., 2003), or multilocus sequence typing (MLST; Enright et al., 2000). Phylogenetic analyses were conducted as described by Kuhn et al. (2006) using whole genome sequences of 30 strains available in public databases (NCBI) or obtained from own bovine strains. The transfer of *S. aureus* between cattle and humans was studied based on the spa types. They resulted from the present study (cattle) or from the study

by Grundmann et al. (2010) who mapped the geographic distribution of dominant types causing invasive human infections in Europe.

Results: All the analyzed strains could be allocated to the 6 genotypes GTB, GTC, GTF, GTI, GTR, and GTOG. Comparing the 3 subtyping methods, RS-PCR showed the highest resolution followed by spa typing and MLST. The associations among the methods were unsatisfactory except for GTB and GTC. *S. aureus* GTB is positive for CC8 (MLST) in 99% of the cases and is typically positive for spa type t2953. *S. aureus* GTB is the cattle-adapted subtype of CC8. *S. aureus* GTC was always positive for t529 and was typically positive for CC705. The link among the 3 methods for GTR and the remaining genotypes was poor. Bovine *S. aureus* is highly clonal and a few clones predominate. Animal *S. aureus* always evolve from human strains whereby every human one may be the ancestor of a novel animal-adapted strain. At least in the case of the bovine genotypes GTB, GTC, GTI, and GTR, the zoonotic transfer of *S. aureus* between cattle and humans is very limited and the different hosts are not considered as a source for mutual, spontaneous infections. Spillover events, however, may happen.

Conclusions: The present study allowed assigning bovine strains to 6 genotypes whereby GTB, GTC, and GTR were the most prominent ones. RS-PCR showed the highest typing resolution followed by spa typing and MLST. *S. aureus* GTB is the cattle-adapted subtype of CC8 and GTC is highly conserved (only 1 spa type was observed). For GTR and the other genotypes, the link among the 3 methods was poor. Bovine *S. aureus* is highly clonal and a few clones predominate. Animal strains of *S. aureus* evolve from human-adapted ones; every human strain may be the ancestor of a novel animal strain. The zoonotic transfer of *S. aureus* between cattle and humans is very limited.

Udder Health & Milk Quality

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FIELD EXPERIENCE WITH A POLYVALENT MASTITIS VACCINE IN A COMERCIAL DAIRY FARM INFECTED WITH STAPHYLOCOCCUS AUREUS

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Objectives: The objective of this poster is to show the efficacy of a mastitis vaccine (STARTVAC⁰) along with management practices to reduce spreading and control *Staphylococcus aureus* in a commercial dairy farm with high prevalence of infection by *S. aureus*

Materials and Methods: The farm is located in Spain milking 600 cows 3 times. Cows in lactation are housed in pens with freestalls bedded with recycled dry manure and environmental conditions are very good, with very low clinical mastitis rate (< 2% monthly). However, the farm has been fighting against *Staph aureus* since 5 years, with high BTSCC and high prevalence of infection. Despite checking the healthy groups by CMT, culturing and segregating *Staph* positive animals many times, and cheking fresh animals after calving by culture, new positive animals appeared after in the healthy group. In July 2014, the farm had problems cleaning and sanitizing the milking machine and we experienced a dramatic outbreak in new cases (64). In February 2015, all the animals in the healthy groups were sampled for PCR twice in a week time difference and we segregated *Staph aureus* positive animals once again. Fresh animals are sampled by PCR one week after calving in the fresh group and positives are segregated. At the same time, we vaccinated all the lactating animals, dry cows and heifers two months before calving

and and revaccinated 21 days. After that, the whole farm has been vaccinated each 3 months. The farm is making monthly SCC test at cow level and we used these data to calculate some key performance indicators related to dynamics of infection, as % of chronic cows, new infection risk, and average linear score

Results: The farm udder health has been improving and only one new case of *Staphylococcus aureus* has appeared since we implemented vaccination and it was in a fresh heifer last August. As a result, BTSCC is decreasing day by day, with less chronic animals (the percentage of chronic cows has been reduced from 27% to 17%), lower new infection risk (the new infection risk is 3% lower on average than in the same period before vaccination) and lower average linear score (the LS has go down from 3,3 to 2,7).

Conclusions: Along with other management practices to control *Staphylococcus aureus*, vaccination in this farm is having a great impact contributing to minimize spreading of *S. aureus* within the herd, improving udder health and reducing Bulk Tank Somatic Cell Count.

Udder Health & Milk Quality

P04-004-222

Development of real time PCR tools for bovine mastitis etiological diagnosis and herd management

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Objectives: Bovine mastitis causes large economical losses worldwide. Identification of mastitis associated-bacteria is a required step to set up adapted dairy farm management. The use of microbiological cultures and biochemical tests for bacterial identification presents several drawbacks: time-consuming, difficult to automate, false-negative results (due to the presence of bacterial growth inhibitors or residual antibiotics), potential miss-identification and is not adapted to composite milk samples analyses. DNA-based diagnosis ie real-time Polymerase Chain Reaction (qPCR) is the best fit tool to detect and quantify major mastitis causing germs.

Materials and Methods: Based on bacterial prevalence in French dairy farms, qPCR assays (Bio-T[®] milk qPCR line) were developed to detect major and minor mastitis causing bacteria as for example *Staphylococcus aureus*, *Staphylococcus spp*, *Streptococcus uberis*, *Streptococcus dysgalactiae* and *Enterobacteriaceae*.

Each qPCR assay was designed as duplex detecting i) the bacteria of interest and ii) an exogenous internal positive control, moreover a standard positive control was developed to enable targeted bacteria quantification too. Such individual qPCR assay format (1 bacterial target / qPCR) enables a flexible tailor-made approach to screen independently from 1 to 12 bacterial targets, depending on the sample type, geographic area or epidemiological context. This format is adapted to selectively target bacteria of interest and avoid thorough screening of numerous pathogens including minor interest germs.

Results: We first report on the experimental inclusivity-exclusivity data, qPCR limit of detection and qPCR efficiency for each target using both purified DNA (genomic DNA, standard plasmid DNAs), spiked and field milk samples. Then, to complete experimental validation, an epidemiological study was done, comparing somatic cell counts, conventional bacterial identification and qPCR using our in house

designs for 5 targets (major mastitis pathogens). 620 quarter milk and 12 bulk tank milk samples were collected from 12 dairy farms deriving from 4 different French regions (3 herds / zone): about 10-15 cows per herd (almost 20% of the herd) including 1-2 control milk cows. First results showed a good correlation between conventional identification method and qPCR data for 66% of milk samples. Besides, this study indicated that qPCR assays enabled to detect a major mastitis causing pathogen for 20% of milk samples tested negative using bacteriological approach and most interestingly qPCR enable to diagnose 48% of milk samples assessed "contaminated" using microbiological cultures.

Finally, to detail further whether qPCR assays could help with herd etiological diagnosis, a study was launched using composite milk samples including bulk tank milks. Indeed, 300 composite milk samples were collected from 60 different herds from Brittany. To get an overview of the herd status, additionally to bulk tank milk, 4 classes of lactating cows were selected for sampling: 1) first calf-heifers (<1 month calving), 2) multiparous cows (<1 month calving), 3) chronically infected cows and 4) dry cows.

Conclusions: This approach is the most adapted since it is difficult and expensive to analyze quarter or even individual cow milk requiring to sample a significant cow number (15 to 35% of the herd) in a limited time laps. qPCR analysis are currently ongoing.

These data confirmed qPCR assay as a good alternative to bacteriological approach: less cost-effective, enabling high throughput analyses and usable for composite milks diagnosis which is a blocking step for herd diagnosis.

Udder Health & Milk Quality

P04-004-223

CASE STUDY: Incidence of Mastitis before and after Vaccination at a Farm in Northern Spain

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Objectives: The objective of this paper is to document the results obtained from one field experience using a vaccine for mastitis (STARTVAC®, LABORATORIOS HIPRA, S.A. SPAIN) , to assess its efficacy at a dairy cattle farm with 140 cows in northern Spain.

Materials and Methods: 120 lactating cows and 20 dry cows in a farm with 2 milking robots were used for this trial.

The main mastitis related pathogens were: Staphylococcus aureus, Escherichia coli and Streptococcus uberis. 1 dose (2ml) of the vaccine was administered to all cows the same day. 21 days later all animals were revaccinated.

Milk production and bulk tank milk somatic cell count were evaluated every two days one year before and after vaccination. New infections rate, cure rate and % of chronic cows were analyzed, during lactation and at dry off, using the individual Somatic Cell Count (SCC) data from the Monthly Milk Record . As agreed by the scientific community, the threshold of 200,000cells/ml was established in order to differentiate healthy udders from infected ones.

Results: Milk yield per cow and day was 20L before vaccination and 29.22L after vaccination. Milk yield per lactating cow and day was 31L before vaccination and 32L after vaccination.

The average SCC before vaccination was 236,000 cells/ml and after vaccination it was 195,000 cells/ml.

New infections decreased in first lactation cows from 8,45% to 5.25%. In adult cows, new infections decreased from 15% to 8.25% and, at dry-off, they decreased from 20% to 8.5%.

The cure rate at lactation in heifers rose from 42.6% before vaccination to 60% after vaccination but in adult cows this trend was the opposite, from 36.6% to 30%. At dry-off the cure rate raised from 38% before vaccination to 91% after it.

Finally, the percentage of chronic cows went down from 6% to 3.5% in heifers, from 20% to 16.5% in adult cows and from 17% to 2.5% at dry-off.

Conclusions: Vaccination was successful in this farm. Milk production and cure rate at lactation, in heifers and at dry-off increased after vaccination. New infections decreased after vaccination and cure rate in heifers and at dry- off improved.

Udder Health & Milk Quality

P04-004-224

Effectiveness of tylosin five-day systemic injection in dry cow therapy

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Objectives: Staphylococcus aureus (SA) and Esculin positive streptococcus (E-strep) including Streptococcus uberis are gram-positive difficult-to-treat mastitis organisms. They also cause subclinical mastitis without clear symptoms but with high somatic cell counts (SCC). Dry period is considered to be important for chronic mastitis treatment period, and systemic treatment with tylosin injection is popularly conducted as dry cow therapy (DCT), especially for controlling SA mastitis. The objective of this study was to examine the effectiveness against subclinical mastitis with tylosin (Tylan200) five-day systemic injection as DCT.

Materials and Methods: In 11 commercial dairy farms in Japan, 331 cows which were 7 days prior to drying-off (totally 1214 quarters with no clinical symptoms), were checked by California mastitis test. The quarter milk samples showing a strong positive reaction were cultured for microbiological examination and measured for SCC. The quarters with particular gram-positive organism (SA, E-strep, Coagulase-negative staphylococci (CNS)) were enrolled in the treatment trial. Cows having target quarters received 5-day Tylan200 injection intramuscularly (30ml per day) accompanied by cefapirine intramammary infusion. Teat sealants were not used. Quarter milk samples were collected at 1 and 2 weeks after calving, and served for microbiological examination and SCC. These cows were observed for 30 days after calving for clinical mastitis symptoms.

Results: The positive rates of each organism were as follows: SA 0.9% (11/1200); E-strep 1.8% (22/1200); CNS 1.4% (17/1200). The samples with contamination were eliminated from the analysis. Bacterial cure was defined as negative bacteria culture on both milk sampling after calving, and without any clinical mastitis until 30th day in milk. The quarters failing to complete sampling were also eliminated. The cure rates of each organism were as follows: SA 66.7% (6/9); E-strep 66.7% (8/12); CNS 100% (2/2). No significant differences were found for the cure rates between the 3 groups.

Conclusions: Gram-positive organism cure rates of Tylan200 injection DCT reported by other groups were 80% and 87%. Bacterial species identification was not clear in these reports, therefore this is first report to research the effectiveness of 5-day Tylan200 systemic injection for specific difficult-to-treat organisms. The cure rates of SA and E-strep subclinical mastitis were both 66.7% in this study. It is suggested that Tylan200 is effective to use in DCT for these difficult-to-treat gram-positive organism infections. Since the number of samples in this study was not enough, further studies are needed to validate this effect in other dairy herds.

Udder Health & Milk Quality

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Udder health in a dairy herd using an off-licence administration protocol of vaccine containing *Escherichia coli* (J5) and *Staphylococcus aureus* (CP8).

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Objectives: This retrospective study aimed to describe udder health parameters in a single dairy herd before and after vaccination with *Escherichia coli* (J5) and *Staphylococcus aureus* (CP8) (Startvac; Laboratorios Hipra, Girona). The licensed programme involves administration of targeted periparturient doses. This protocol can be impractical to deliver on some units, leading to adoption of a simpler programme in which paired starting doses precede whole herd quarterly repeat vaccination. The objective of this study was to identify trends in mastitis cases and somatic cell count, looking for associations with use of the off-licence vaccination programme.

Materials and Methods: A dairy unit was recruited comprised of five hundred pedigree Holstein cows, permanently housed, with an even calving pattern throughout the year. A protocol had been implemented in which two vaccine doses (Startvac) were administered to the whole herd at a three week interval, followed by repeat doses at three month intervals. Clinical mastitis data was recorded contemporaneously by the farm staff. Individual cow level somatic cell counts were sampled every four weeks (Cattle Information Service, Telford). These three areas were examined for twelve months preceding the use of the vaccine and thirty months following the commencement of vaccination. Mastitis data was collated in to monthly case rate and, along with somatic cell count values, underwent descriptive analysis (Excel 2010, Microsoft Corporation). The dataset was then examined by univariate analysis of monthly medians (Mann-Whitney) and monthly means (two sample t-test after Johnson's transformation to account for non-parametric distribution) for the periods of the study preceding vaccination and following commencement of the programme (Minitab 17.1.0, Minitab inc.).

Results: Clinical case rate for the period preceding vaccination had a mean of 3.85 index quarter cases per hundred cows per month (95% CI 3.34, 4.35) and 4.38 total quarter cases per hundred cows per month (95% CI 3.80, 4.97). This case rate was not statistically different for the twelve month period immediately after commencement of vaccination, with index cases of 3.73 per hundred cows per month (95% CI 2.86, 4.61) and total case rate of 4.53 per hundred cows per month (95% CI 3.58, 5.47). The final eighteen months of the dataset had a mean case rate of 2.99 index quarter cases per hundred cows per month (95% CI 2.55, 3.44) and total case rate of 3.41 per hundred cows per month (95% CI 2.88, 3.94). The index case rate in the final period of the study was significantly lower than the period preceding vaccination by 0.86 cases

per hundred cows per month ($p=0.011$). Total cases were reduced by 0.97 cases between these two periods ($p=0.014$). Median case rates for these periods were similarly reduced, with a reduction of 0.85 index cases per hundred cows per month ($p=0.014$) and a reduction of 0.95 total cases per hundred cows per month ($p=0.016$). This equates to a reduction of approximately fifty-one index cases (fifty-seven total cases) for the herd per year.

Somatic cell count was relatively stable over the period of the study. No statistically significant differences in cell count were observed between the periods prior to vaccination and following commencement of the programme.

Conclusions: A reduction in mastitis rate started nine months after commencement of vaccination and continued over the remainder of the study. Multivariate analysis was beyond the scope of this dataset, so it was not possible to draw firm conclusions about causality. However, other than the vaccination programme, no major changes were made during the study period that would influence udder health. The temporal trend corresponds with previous reports of efficacy for the 'off-licence' vaccination protocol and supports earlier work suggesting that off-licence quarterly vaccination (Startvac) reduces the impact of intramammary infection in dairy cows.

Udder Health & Milk Quality

P04-004-226

Prevalence Of Milk Pathogens From Bulk Tank Milk In Azorean Pastures

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Objectives: To our knowledge no prevalence's of a milk pathogens wide range, using multiplex qPCR technique from pastures of San Miguel Island (Azores, Portugal) were described before. The aim of the present study was to determine the apparent prevalence of a large wide of milk pathogens from BTM of Azorean dairy herds using a multiplex PCR method.

Materials and Methods: From all regions of San Miguel- Azores, 92 dairy herds considering a total of 5520 adult cows were used. These herds represented 5.0% (1833) of total dairy farms according data officially reported (2014) in San Miguel Island.

A BTM sample from each selected herds was collected using the (Startcheck®) sampling kit (HIPRA, Spain), according the manufacturer instructions, in order to determine the presence of coagulase-negative staphylococci (CNS), *Escherichia coli*, *Staphylococcus aureus*, *Corynebacterium bovis*, *Enterococcus* spp., *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Actinomyces pyogenes*/*Peptostreptococcus indolicus*, *Klebsiella* spp., *Streptococcus agalactiae* and *Serratia marcescens*.

The extraction and amplification of the DNA were performed using a real time PCR (PathoProof™ Mastitis Complete-12 Kit; Thermo Fisher Scientific Inc., MA USA), with some modifications. A qPCR positive result was recorded when cycle threshold values were ≤ 37 and a sigmoidal amplification plot was obtained.

Results: In overall, all the 11 species/groups were detected by the qPCR assay. The mean number of bacteria species or groups for BTM was 5.6 (confidence interval 95% between 5.2 and 5.9). The group of

CNS were detected in all (100%) BTM samples. *E. coli* (75.0%), *S. aureus* (62.0%), *Corynebacterium bovis* (57.6%), *Enterococcus* spp. (55.4%), more than 50% of the herds. The remaining milk pathogens, *Streptococcus uberis*, *Actinomyces pyogenes/Peptostreptococcus indolicus*, *Klebsiella* spp., *Streptococcus agalactiae* and *Serratia marcescens* were present in 41.3%, 41.3%, 37.0%, 32.6% and 3.3% of the BTM samples, respectively.

Conclusions: We have presented baseline figures for 11 mastitis pathogens/groups in BTM samples from Azorean dairy herds. Both environmental or contagious bacteria, CNS, *E. coli* and *S. aureus* are the pathogens more commonly found. These results emphasize once again the importance of implementing mastitis control plans for the control of milk quality in the Azorean dairy herds.

Udder Health & Milk Quality

P04-004-227

The effectiveness of different pre- and post-milking teat disinfectants against *Prototheca zopfii* algae

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Objectives: Since the first identification, the *Prototheca zopfii* became the 4th most common mastitis pathogen in Hungary. Without an effective treatment the only way to control this disease is prevention, and in this, the before and after milking teat disinfection plays a major role.

Materials and Methods: For our experiment we collected four different pre dipping and nine different post dipping products from Hungarian dairy herds. We wanted to provide the exact same conditions every time, to standardize the experiment, therefore we made artificial teats (fingers of plastic gloves filled with cotton swabs) and after proper disinfection these teats were used. Every teat was dipped into a *Prototheca zopfii* broth, and the disinfectants were used on wet and dry teats as well. After the necessary waiting time, the teats were pushed to Sabouraud agar plates. After 48 hours of incubation on 37°C the number of algae colonies was counted on the agar plates.

Results: We compared four different pre dipping products. The polyhexamethylene biguanide (PHMB) was totally ineffective, while the chlorhexidine was much more effective but didn't kill all the algae on the teat. In both cases the wet teats has much worse results than the dry teats. The didecyl-n methyl ammonium chloride and the PVP-iodine based products were able to kill all the algae on the wet and the dry teats too. From the nine post dipping products four contained lactic acid in different concentrations. One of them was effective while the other three has moderate effectiveness. The five iodine based post dipping products has better effectiveness, especially on dry teats, but slight differences could be seen according to the amount of the active material concentration of the products.

Conclusions: In case of teat disinfection the agent is only one of the things which have an effect on the result. In the literature usually the iodine based products are advised against the algae and we found that most of the time they really work. But these are not the only good active materials, and other factors can play a major role in the effectiveness of the disinfection. The proper dipping, and the leaving of unnecessary teat washing also can be important.

Udder Health & Milk Quality

P04-004-228

Pathogens Agents of the Udder isolated Milk Reception Boat of Goats with Mastitis The Municipality Oof Tanhuato Michoacán

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Objectives: Identify the pathogens agents of the udder isolated milk reception boat of goats with mastitis the municipality of Tanhuato, Michoacán

Identify the presence of *Staphylococcus aureus* as the main causative agent of mastitis in goats Tanhuato Township, Michoacán

Materials and Methods: The number of sample units (n = 49 stables) was calculated using a random model with proportional distribution function of the system characterization by small-scale dairy and major conglomerates. Sampling within herds was per cluster, ie a milk sample pot of receipt of each production unit was taken. the sampling of milk using for this test tubes sterilized sealing plug in which the necessary identification previously stable are scored. The test tube is placed inclined to keep out dirt. The sampling was made directly from the milk storage tanks. The sample tube was filled with two thirds at most. Finally, the sample tubes were placed on a rack placed in a sealed cooler for further transport to the laboratory of bacteriology of USAD of the Faculty of Veterinary Medicine and Animal Husbandry for immediate processing or refrigeration at 4 ° C. The processing of the milk samples obtained was held on sampling or the next day. Samples were seeded in 110 staphylococcal agar, azide blood agar and Mc Conkey agar. Then, the agar plates were incubated at 37 ° C and examined after 24 and 48 hours. The isolates were identified by colonial morphology, Gram stain, catalase test, coagulase test and the test of mannitol and gelatin. A isolates Mc Conkey agar, they were made corresponding biochemical tests for identification. The tests used were: Urea, citrate, SIM (Sulfide Indole Motility) test, Agar triple-sugar-iron for its acronym in English TSI, Middle MRPV (methyl red Voges-Proskauer).

Results: 49 samples of the boat receiving equal number of dairy herds goats, of which a total of 61 bacterial strains (100%) were isolated were collected. Of these, 29 (47.54%) were Gram-positive pathogens, while 32 (52.46%) Gram-negative pathogens. On the results of bacteriological analysis of the milk samples obtained in this study (Table 1), the staphylococci were identified as the main etiologic agents Gram-positive mastitis, among which the *S. aureus* as the main causal agent 21 insulation, equivalent to 34.43%, as well as coagulase negatives which mainly found pathogen was *Staphylococcus epidermidis* with 8 isolates representing 13.11%, results appear to be similar to an investigation by Ferrer, et al (1993) who obtained 12 cases, equivalent to 11.5% of *Staphylococcus aureus* and 46 equivalent to 44% divided into agents coagulase negative cases.

Conclusions: It is concluded that *Staphylococcus aureus* was the main pathogen found in samples of milk pot receiving goats Tanhuato municipality, Michoacan, followed by *Proteus mirabilis*, coagulase Negative (*Staphylococcus epidermidis*) and agents Gram negative pathogens: *Citrobacter intermedium*, *Proteus rettgeri*, *liquefaciens* *Serratia*, *Alcaligenes faecalis* and *Citrobacter freundii*.

Udder Health & Milk Quality

P04-004-229

Determination of the quality of Bovine Milk produced in the municipality of TARÍMBARO MICHOACÁN

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Objectives: The aim of this study was to determine the quality of bovine milk produced in the municipality of Tarimbaro Michoacan, through California test, Lactoscan and Microbiological Control.

Determine the percentage of butterfat, protein, and colony forming units/ml in milk samples obtained Tarimbaro municipality, Michoacan.

Materials and Methods: The study was conducted from september of 2012 to may of 2013 in the municipality of Tarimbaro, Michoacan. 100 dairy herds of the same number of production units of municipality Tarimbaro, Michoacan during the morning milking were sampled. To do this, a milk sample pot of receipt of each herd, previously mixed with a tablespoon placing the sample in a plastic pallet for California test was taken. Then the other part of the sample is poured into a glass jar previously sterilized. Subsequently, we proceeded to place samples in a sealed cooler at a temperature of 4 °C for transport immediately bacteriology laboratory of the Faculty of Veterinary Medicine UMSNH for processing. Samples were analyzed by casting technique plate, both for coliform and for mesophilic aerobic bacteria (BMA).

Results: It was found that 94% of the samples were negative, and 6% grade traces with test of California, that is of excellent quality according to international standards. Regarding the determination of butterfat in g/L with Lactoscan analyzer based on the NMX-F700-COFOCALEC 2004 standard, it was found that 69% of the samples corresponded to class A, 5% to class B, 6% to class C, while the remaining milk (20%) was found outside the norm. As for the results of bacteriological analysis for BMA, these are also interpreted according to the NMX COFOCALEC F-700, (2004), found that 46% of the samples corresponded to class 1, ≤100,000 CFU/ml, 11% to Class 2, 12% in Class 3, 7% to the Class 4, while the rest (24%) containing more than 1,200,000 CFU/ml, were classified outside the provisions of this standard.

Conclusions: It is concluded that the milk produced in the town of Tarimbaro, Michoacan is good quality, and to comply with the parameters established nationally and internationally. Making it suitable for consumption by the public consumer without risk.

Udder Health & Milk Quality

P04-004-230

Bacterial profiles and risk factors of intramammary infections in french dairy herds with high bulk tank milk somatic cell countOlivier Salat ^{1,*}

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Objectives: High milk somatic cell counts keep a major concern in dairy herds. In such herds, a rational process must be implemented to understand the infection dynamics. Identification of intramammary infection risk factors is just a step. Diagnosing the aetiology of intramammary infection makes up the other step, and it is critical to understand what happens in these dairy farms

Materials and Methods: This study presents bacterial profiles and risk factors reported in 66 dairy herds with concern about high bulk tank milk somatic cell count. All these farms were visited to evaluate management practices, housing conditions and milking routines. Dry cow routines and treatment protocols were also recorded. During the visit, a CMT (Californian Mastitis Test) was performed on the cows with high somatic cell counts (≥ 300 000 cells/ml) and the positive quarters were aseptically sampled for culture. When the same pathogen was frequently isolated, susceptibility to six antimicrobials was looked up. When the isolated strains showed the same antibioresistance pattern, we concluded that it was the same contagious strain of the pathogen.

Results: In about 40% of the herds, bacteriological exams revealed that one contagious strain was responsible of more than 70% of intramammary infections, and *Staphylococcus aureus* was isolated in more than ¾ of these herds. Strains of *Streptococcus uberis* (2 herds), *Streptococcus dysgalactiae* (2 herds), *Staphylococcus caagulase negative* (1 herd) and *Escherichia coli* (1 herd) were also identified as contagious bacteria. In the other herds, we found a variety of pathogens, with *Staphylococcus aureus* and *Streptococcus uberis* as the most prevalent and equally responsible of more than one half of intramammary infections.

The main risk factors of Intramammary infections were poor milking hygiene, no early detection of clinical mastitis and no prevention of contamination by milking unit. There was no specific risk factor in the herds where contagious bacteria were isolated

Conclusions: When an effectively contagious bacteria circulates within a herd, which is found in about 40% of dairy herds with high bulk tank milk somatic cell counts, the main factor explaining intramammary infections is the contagious potential of the pathogen.

Udder Health & Milk Quality

P04-004-231

Trends in U.S. Milk Quality Based on Bulk-Tank Somatic Cell CountsJason Lombard ^{1,*}Christine Kopral ¹Kathe Bjork ¹

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Objectives: Bulk-tank somatic cell counts (BTSCCs) are used as one measure of milk quality. The maximum BTSCC allowed in milk varies in countries around the world. The current limit in the United States is 750,000 cells/mL, while other countries such as the EU have limits of 400,000 cell/mL. BTSCCs are important in international trade since exporting countries are required to meet the limits set by importing countries. In 2012, the EU implemented regulations that require milk products exported to the EU have a maximum BTSCC of 400,000 cells/mL. The objective of this study was to evaluate changes in bulk-tank somatic cell counts (BTSCC) from 2000 to 2014.

Materials and Methods: BTSCC data from four Federal Milk Marketing Orders (FMOs), representing about half of the milk marketed in the United States, were used to evaluate changes in BTSCCs. The four FMOs were Mideast, Upper Midwest, Central and Southwest. Data were

collected monthly and included herd identification, FMO, kilograms of milk shipped, and official BTSCCs. These data represent all milk shipped through the four FMOs, and conclusions apply only to this population of shipments. Because this study was a census, no estimates of sampling variability were calculated. A milk-weighted, 3-month geometric mean BTSCC was calculated and summed for all herds in each FMO and for all herds. SAS software was used for all calculations.

Results: In 2014, 45.0 billion kg of milk from 284,528 shipments representing 26,424 producers were monitored. Monitored milk represented 48.1% of the 93.6 billion kg of milk produced in the United States in 2014. The milk-weighted mean BTSCC decreased from 296,000 cells/mL in 2000 to 193,000 cells/mL in 2014, while the producer-weighted mean BTSCC decreased from 320,000 to 229,000 cells/mL during the same period. The Upper Midwest FMO accounted for approximately 45% of milk monitored on an annual basis. The Mideast FMO had the lowest BTSCCs for 13 of the 15 years evaluated. Seasonal variations in BTSCCs were consistent from 2000 to 2014, with the highest counts occurring in July through September. The summer increase in BTSCCs over baseline, however, has decreased from approximately 60,000 cells/mL to 35,000 cells/mL over the time period. The percentage of milk shipped from the 4 FMOs with BTSCCs of less than 400,000 cells/mL increased from 74.8 to 95.5%, while the percentage of shipments with counts less than 400,000 cells/mL increased from 65.0 to 86.8%.

Conclusions: BTSCCs in the United States have decreased approximately 35% since 2000. Many factors are likely responsible for these improvements in milk quality, e.g., producer motivation in the form of bonuses for providing quality milk, milk-quality regulations of countries that import U.S. dairy products, and an emphasis on improving milking procedures.

Udder Health & Milk Quality

P04-004-232

The production effects and economic evaluation of vaccination against *Staphylococcus* and *E. coli* mastitis on a large-scale Hungarian dairy farm

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Objectives: The mastitis has always been responsible for one of the most significant production losses in the dairy industry. The losses caused by mastitis were estimated to be more than € 50 million in 2011 (about € 200 per cow on average) in the Hungarian dairy cattle sector, and according to the recent surveys, the coagulase-negative *Staphylococci* (CNS) and the *Staphylococcus aureus* are responsible for most mastitis cases. The aim of our study was to present the production effects and the economic evaluation of mastitis vaccination against *S. aureus*, CNS spp. and *Escherichia coli* in a large-scale Holstein-Friesian dairy herd.

Materials and Methods: The study was carried out in a 700-cow Hungarian dairy herd, in which a large number of mastitis cases, mainly caused by *S. aureus* and CNS spp., resulted in high milk somatic cell counts (SCC), high culling rate in cows and significant treatment costs. The mastitis vaccination (Startvac®, Hipra) was started in 2010 on the farm. We surveyed the major milk production indices (average lactation and daily milk production, monthly SCC) and udder health parameters (occurrence and distribution of mastitis pathogens, number of clinical mastitis cases and udder treatments, mortality and culling rate in cows

due to mastitis) and their economic impact on the farm between 2006 and 2014. Thus, we were able to compare the losses caused by clinical mastitis (cost of curative treatments, discarded milk and cow replacement [mortality and culling]) in the 4-year-long period before the vaccination (2006-2009) with those in the 4-year-long period after the entire cow population was vaccinated (2011-2014) in order to conduct the cost-benefit analysis of the mastitis vaccination. In the economic analysis the reduction of losses due to clinical mastitis was considered as benefit and the expenditures of vaccination were taken into account as cost. All the benefits and costs occurred over the 9 years of the survey period were adjusted for the general price level in 2014.

Results: After vaccination the incidence and proportion of the CNS spp. in the mastitis cases has moderately dropped, nevertheless, the clinical mastitis cases caused by *E. coli* have disappeared and the occurrence of *S. aureus* remained low throughout the whole vaccination period. The average daily number of cows with clinical mastitis has significantly decreased after 2010 from over 30 to between 5 and 10 cows a day. So did the monthly number of mastitis treatments: from more than 800 in 2006 to less than 300 in 2014. Thus, the quantity of discarded milk has also greatly decreased (from 6.1% to 1.2% of the total milk production), and so did the curative mastitis treatment cost (from € 18.3 to € 10 per cow). The monthly average SCC has slightly diminished after 2010, but no significant decrease could be observed. The mastitis vaccination had no impact on cow replacement owing to mastitis.

At 2014 price level the cost-benefit analysis of mastitis vaccination shows that the yearly loss caused by clinical mastitis in the 4-year-long period before the vaccination (2006-2009) was € 206.3 per cow which decreased to an annual amount of € 138.0 between 2011 and 2014 when all the cows were vaccinated. It included the significant decrease in the cost of discarded milk and mastitis treatments, however, that of premature disposal increased because of the enlarged heifer raising costs. The mastitis vaccination yielded € 50.7 average annual profit (decrease in loss – cost of vaccination) per cow between 2011 and 2014. The vaccination as an investment had 3.37 benefit-cost ratio and 236.8% ROI.

Conclusions: The findings of the case study show that the mastitis vaccination significantly reduced the occurrence of clinical mastitis cases caused by *S. aureus*, CNS spp. and *E. coli* in the herd. According to the result of the economic analysis the mastitis vaccination (Startvac®, Hipra) was profitable on the surveyed farm, as the decrease in the loss due to mastitis coming from the improved udder health parameters exceeded the mastitis vaccination costs.

Udder Health & Milk Quality

P04-004-233

Disagreement between milk pathogens prevalence in segregated milking cows and bulk tank milk in Azorean pastures

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Objectives: The aim of the present study was to determine the apparent prevalence of a large wide of milk pathogens from BTM using a multiplex PCR method and compare it with the pooled samples from mastitic cows segregated at milking time from Azorean dairy herds.

Materials and Methods: From all regions of San Miguel- Azores, 92 dairy herds considering a total of 5520 adult cows were used.

The separation of mastitic cows, due to the presence of clinical mastitis (untreated or in treatment) or subclinical mastitis with high SCC detected by California Mastitis Test, at milking time was considered for herd inclusion on the present study. A milk pooled sample from all quarters of these cows (mean = 3) were collected in each farm. In simultaneous, after milking all cows, a sample was collected from BMT.

All final samples were collected using the Startcheck® sampling kit (HIPRA, Spain), according the manufacturer instructions, in order to test the presence of coagulase-negative staphylococci (CNS), *Escherichia coli*, *S. aureus*, *Corynebacterium bovis*, *Enterococcus* spp., *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Actinomyces pyogenes* / *Peptostreptococcus indolicus*, *Klebsiella* spp., *Streptococcus agalactiae* and *Serratia marcescens*.

The extraction and amplification of the DNA were performed using a real time PCR (PathoProof™ Mastitis Complete-12 Kit; Thermo Fisher Scientific Inc., MA USA), with some modifications. A qPCR positive result was recorded when cycle threshold values were ≤ 37 and a sigmoidal amplification plot was obtained.

Results: In average, each farm presented 4.6 (CI95% from 4.3 to 4.9) and 5.6 (CI95% from 5.2 to 5.9) bacteria species/group from SMC and BMT respectively. Coagulase-negative staphylococci, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus* spp., *Corynebacterium bovis* and *Streptococcus* spp. presented the higher apparent prevalence on both samples type (upper 95% IC > 50%). A high correlation of apparent prevalence from each pathogen between SMC and BMT samples were observed (Rho = 0.90; n = 11; P < 0.01) inter-herds. However, the agreements between these apparent prevalences, intra-herd, were always low (Kappa coefficient < 0.70).

Conclusions: In conclusion, high prevalences of bacteria species/groups were detected in BTM from Azorean pastures. Although the segregation of cows may be a potentially favorable practice, their number on each farm seem to be too small to be representative and consequently not an indicator of agents in BTM on the same herd. In consequence, a holistic approach on mastitis diagnosis, treatment and control should be considered in Azorean dairy farms.

Udder Health & Milk Quality

P04-004-234

Selective dry cow therapy in the UK using OrbeSeal™

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Objectives: Selective dry cow therapy is increasingly becoming important in UK dairy herds. The decision about whether a cow should receive antibiotic dry cow therapy (ADCT) in addition to an internal teat sealant or an internal teat sealant alone should be based on farm information including somatic cell count recordings, clinical mastitis case rate and knowledge of the predominant mastitis pathogens on the farm.

Materials and Methods: At Zoetis we believe it is important that selective dry cow therapy using OrbeSeal is performed correctly using all the farm information available. The flowchart below is designed to offer a guide to decision making for determining the appropriate dry cow therapy at the herd, group and then individual animal level.

Stage 1 - Herd level

Do you record clinical mastitis cases in an accessible format to analyse?

Have you performed bacteriology to understand the pathogens causing clinical and subclinical mastitis on your farm?

Do you carry out monthly individual cell count recordings?

Yes to all of the above - you can now determine which groups of cows will be eligible for selective dry cow therapy

No to any of the above - based on herd level factors you do not have enough information to determine cow groups for performing selective DCT, Discussions are required between vet/farmer and milk purchaser about recording farm data and carrying out milk sampling.

Results:

Stage 2 - Group level

Split herds into low, medium and high SCC to identify groups within the herds for dry cow therapy.

Stage 3 - Individual cow decision making

Once cows determined appropriate for selective dry cow therapy have been identified, a final check should be carried out to make sure the cow is not of high risk for mastitis during the subsequent lactation.

Individual cow risk factors for mastitis

- Environment: high bacterial populations at the teat end
- Production: high milk production across lactation, poor or delayed formation of keratin plug
- Drying off: increasing parity, high milk production at drying off, gradual method of dry off, poor hygiene and cleanliness at dry off, milk leakage at dry off, poor teat end integrity (eg damage, hyperkeratosis)

Conclusions: Selective dry cow therapy is important in UK dairy herds to reduce the usage of antibiotics and on some farms as part of a mastitis control programme. OrbeSeal prevents mastitis acquired during the dry period and is therefore a vital tool in mastitis control, whether used with or without an antibiotic dry cow product. The use of OrbeSeal alone should be targeted at cows which have a reduced risk of having a mastitis infection at dry off. OrbeSeal should always be infused in a clean and hygienic manner to reduce the risk of iatrogenic infection.

Comments: To discuss the use of OrbeSeal either alone or with an antibiotic dry cow product further, please contact a member of the Zoetis Ruminant Technical team or email: customersupportUK@zoetis.com.

Young Stock

P04-004-130

Serum Biochemical Profile Of Healthy Murrah Buffalo Calves In The Neonatal Period

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Objectives: The present study aims to establish a serum biochemical profile of healthy Murrah buffalo calves, from birth to 30 days of age, and also to evaluate indirectly the passive immunity transfer on those animals

Materials and Methods: The research project was evaluated by the Ethics Committee on Animal Use (CEUA) of FCAV / UNESP / Jaboticabal Campus and approved under protocol number 010028/2014. Thirty five healthy Murrah buffalo calves born from healthy dams in eutocic births were evaluated. The research was made in São Paulo State - Brazil. Blood samples for blood serum separation were collected after birth (M0), and with 24h (M1), 48h (M2) and 72h (M3) of age, and after 7 (M4), 14 (M5), 21 (M6) and 30 (M7) days of life to determine the serum activities of gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and creatine kinase (CK) and serum concentrations of total protein (TP), albumin (Alb), iron (Fe), total calcium (Ca) and phosphorus (P) by spectrophotometry using commercial reagents. The blood samples were centrifuged at 1,000 x g for 10 minutes, and serum samples were frozen at -20°C until analysis.

Results: The results are as follows: GGT (U/L) - M0: 19.1±6.10; M1: 1,421±803; M2: 544±354; M3: 313±177; M4: 153±99.5; M5: 63.0±43.8; M6: 34.6±27.5; M7: 18.1±7.67. ALP (U/L) - M0: 142.5±57.5; M1: 1,272±638; M2: 501±271; M3: 301±140; M4: 207±107; M5: 141±52.5; M6: 115±37.3; M7: 103±42.5. AST (U/L) - M0: 43.7±15.8; M1: 106±17.4; M2: 99.2±16.7; M3: 893±13.9; M4: 77.4±8.90; M5: 74.1±31.3; M6: 69.8±21.1; M7: 70.9±13.0. CK (U/L) - M0: 121±53.1; M1: 274±114; M2: 164±82.6; M3: 136±85.5; M4: 138±47.5; M5: 234±120; M6: 250±122; M7: 255±98.7. TP (g/dL) - M0: 4.80±0.26; M1: 8.10±1.31; M2: 8.22±1.34; M3: 8.14±1.39; M4: 7.88±1.20; M5: 7.56±0.96; M6: 7.21±0.79; M7: 6.77±0.79. Alb (g/dL) - M0: 2.44±0.24; M1: 2.11±0.23; M2: 2.19±0.23; M3: 2.31±0.24; M4: 2.48±0.20; M5: 2.56±0.20; M6: 2.65±0.24; M7: 2.57±0.29. Fe (µg/dL) - M0: 84.7±29.7; M1: 80.7±42.9; M2: 119±58.9; M3: 116±72.3; M4: 148±135; M5: 138±82.1; M6: 191±102; M7: 187±79.8. Ca (mg/dL) - M0: 9.86±1.08; M1: 9.60±1.32; M2: 10.5±1.04; M3: 10.6±1.18; M4: 10.74±1.12; M5: 10.1±1.14; M6: 9.92±1.32; M7: 9.47±1.35. P (mg/dL) - M0: 5.39±0.73; M1: 6.58±1.47; M2: 7.58±1.88; M3: 9.04±1.74; M4: 9.38±1.12; M5: 8.63±0.88; M6: 8.46±0.90; M7: 8.28±1.09.

Conclusions: These results help to establish a biochemical serum profile of healthy Murrah buffalo calves in the neonatal period, aiding in the interpretation of physiological and pathological conditions that may have an impact on the evaluated parameters. GGT activity as well as TP levels in M1 showed results well above of the satisfactory levels for a secure passive immunity transfer from the dams to the calves

Comments: Thanks For FAPESP for the aid Process n.2014/09246-5

Young Stock

P04-004-131

Leukogram And Acute Phase Proteins Concentrations Of Calves With Rotavirus Diarrhea

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Objectives: The aim of the present study was to characterize changes in leukogram and acute phase proteins profile as a result of the occurrence of rotavirus diarrhea in calves throughout the first month of life.

Materials and Methods: Blood and fecal samples were collected from twenty-four Holstein calves allotted in three experimental groups with eight animals each: calves that did not present diarrhea neither rotavirus infection (group A), calves that presented diarrhea, but tested negative for rotavirus in feces (group B), and calves that presented diarrhea and tested positive for rotavirus in feces (group C) before colostrum intake (moment 0) and at 1, 2, 7, 15, 21 and 30 days of age (Experiment 1). Also, whenever the animals presented episodes of diarrhea, blood and fecal samples were collected at 24-hour intervals until the end of clinical signs (Experiment 2). Leukogram was performed in an automatic counter and acute phase proteins in serum were electrophoretically separated in polyacrylamide gels containing sodium dodecyl sulphate (SDS-PAGE); rotavirus in feces was detected by polyacrylamide gel electrophoresis (PAGE). Data from experiment 1 were analyzed by ANOVA and the differences between groups were compared by Tukey's test, considered significant at P<0.05. Data from experiment 2 were subjected to the HSD test.

Results: Total leukocyte, neutrophils, and lymphocyte counts did not differ among groups A, B, and C throughout the first 30 days of life, although, in experiment 2, least square means for total leukocytes and lymphocyte counts were significantly higher in the first day of diarrhea manifestation in group C when compared to group B. In experiment 1 ceruloplasmin and transferrin levels were significantly higher in group C than in groups A and B. Serum concentrations of haptoglobin and α_1 -acid glycoprotein did not differ significantly among groups throughout the experimental period. In the moments of diarrhea manifestation, least square means of ceruloplasmin and transferrin concentrations were higher in group C than in group B, whilst haptoglobin and α_1 -acid glycoprotein concentrations did not differ significantly between groups B and C.

Conclusions: These findings suggest that rotavirus diarrhea caused a more significant tissue damage followed by leukocyte response when compared to other causes of diarrhea affecting calves throughout the first month of life, and that ceruloplasmin may be a marker of rotavirus infection in calves.

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Young Stock

P04-004-132

Assessment of dietary isoquinoline alkaloids in the prevention of cryptosporidiosis in newborn calves

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Objectives: Diarrhea presents a high prevalence in newborn calves and is considered the main cause of mortality in this category. The infection by *Cryptosporidium* spp. is one of the main etiologies of this disease. Specific isoquinoline alkaloids have local anti-inflammatory effects and can contribute for the intestinal integrity. The objective of the study was to evaluate the effectiveness of Sangrovit® CS in the prevention of diarrhea caused by cryptosporidiosis in experimentally inoculated calves, by comparing the intensity and duration of the induced diarrhea.

Materials and Methods: A total of 26 newborn male calves (20 Holstein and 6 crossbred ¾ Holstein X ¼ Gir), were randomly divided into two groups of 13 animals each. Group A received isoquinoline alkaloids (10 g/animal/day of Sangrovit® CS divided in 2 meals/day) from the second meal of the 1st day of life until the 21st day of life. Group B (control group) received milk without any additives. All calves were orally inoculated on the third day of life with 1×10^6 *Cryptosporidium parvum* oocysts. The inoculum was obtained from a field case, amplified in a healthy calf and subsequently purified and kept in the laboratory of the UFMG's Clinic of Ruminants sector. All animals were evaluated daily from the 3rd until the 30th day of life for the occurrence, duration and intensity of diarrhea. In order to determine the fecal consistency, the following score was established: 0 (normal feces), 1 (pasty feces), 2 (watery feces), 3 (profuse diarrhea with watery feces) and 4 (bloody diarrhea). The scores 2, 3 and 4 were characterized diarrheal feces. Hemogasometry was performed each two day during diarrhea. Animals that have base deficit greater than 9 meq/l were hydrated to aid in recovery of the disease, and were considered with prognosis reserved.

Results: In group A, 84.62% of the calves had diarrhea lasting an average of 6.31 ± 6.49 days, while in group B all calves had diarrhea that lasted on average 11.55 ± 5.29 days. In group A, the number of animals that required hydration was 15.38% (2/13), lower ($P < 0.05$) than in group B with 69.23% (9/13). Furthermore, in group A no mortality was observed while in group B 15.38% (2/13) of the animals died in consequence of cryptosporidiosis. The consistency of fecal scores of treated calves were lower during the studied period and feces showed up more solids ($P < 0.05$) during the 6th, 7th and 8th day of diarrhea.

Conclusions: Oral administration of Sangrovit® CS decreased the intensity and duration of diarrhea symptoms and reduced the need to support treatments for clinical recovery as well as the mortality rate of the affected animals.

Young Stock

P04-004-133

Clinical and Hematological findings in Neonate Holstein Heifers fed with whole and cell-free colostrum

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Objectives: The neonatal period is critical for the survival of newborn heifers due to the adaptation to the environmental and immunological immaturity, making them dependent on maternal immune factors transferred by colostrum intake. The hypothesis of this study is that colostrum cells and their products, working with antibodies, can collaborate in the development of the innate and adaptive immune system to reduce the incidence and severity of disease during this period. So, the objective of this research was to evaluate the influence of colostrum cells on the health and dynamics of the hematological profile in heifers during the first 28 days of life.

Materials and Methods: In this study, healthy Holstein heifers (n=19) were selected using a clinical examination between birth and two hours of life. Heifers were distributed in two experimental groups: COL+ (n=9) which received fresh colostrum from their own dams; and group COL- (n=10) which received frozen colostrum containing no viable cells. Both groups received colostrum of the same quality measured by immunoglobulins concentration (70-120g/L – colostrometer and 23-32% - BRIX). Heifers were assessed before colostrum intake – D0; 24-48 hours after birth – D2; 7 days after birth (ab) – D7; 14 days ab – D14; 21 days ab – D21 and 28 days ab – D28. Heifers were given fecal and bronchopneumonia scores, umbilical palpation and hematological profile. Fecal scores were assigned as; 0- normal consistence, 1- pasty, semi-formed; 2- pasty with largest amount of water; or 3- liquid with fecal content adhered in the perineum and tail. Bronchopneumonia was scored using the following parameters: rectal temperature, cough, nasal and ocular secretion and ear position with a score of 0-3 for each based on severity. Calves were assessed as having bronchopneumonia when the sum of these scores was >5. Parametric data were evaluated between groups using an unpaired T test and analysis in time by ANOVA for repeated measures ($P < 0,05$). Qualitative data were presented in absolute values and frequencies and the groups were compared by Chi-square test.

Results: The frequency of diarrhea was high from D7 (COL = 55.6% and COL- = 70%) to D28 (COL+ = 44.4% and COL- = 50.0%) in both groups ($P = 0.418$), however, the COL- had a higher intensity score (typically 3) on D7. Bronchopneumonia was detected just one case in D21 in COL- calf. Umbilical inflammation was detected in 3 heifers at D14 and D28 in COL- calves. COL- heifers had lower numbers of red blood cells (RBC) ($6.5 \pm 0.8 \times 10^6/\mu\text{L}$) and less hemoglobin (HB) ($8.3 \pm 1.4 \text{ g/dL}$) than those from COL+ group ($\text{HE} = 7.2 \pm 0.8 \times 10^6/\mu\text{L}$; $\text{HGB} = 9.6 \pm 1.3$) at D14. The values for RBC, HB and hematocrit (HTC) decreased during this study. Anemia was detected during neonatal period, with higher frequency in COL- (40.0 and 50.0%) than the COL+ group (0.0%) at D21 ($P = 0,03$) and D28 ($P = 0,02$). The hematological profile revealed a peak of white blood cells (WBC) at D7 in both groups. COL+ calves had a gradual decrease in the number of WBC until D21, then an increase at D28. However, COL- group had decreased numbers of WBC through D28. Both groups had gradual decrease in neutrophils, and an increase in lymphocytes (Ly) over the course of the study. However higher numbers of Ly were observed on D7 in COL- calves relative to those receiving maternal cells ($3.8 \times 10^3/\mu\text{L}$ COL+ and $5.4 \times 10^3/\mu\text{L}$, $P = 0.02$).

Conclusions: Thus, we observed that the occurrence of diseases was similar between groups, except for umbilical inflammation. However, diarrheal scores 2 or 3 were observed more frequently in COL-calves. The hematological values of heifers that received cell free colostrum resulted in anemia and leukocytes alteration compatible with inflammatory process.

Comments: This research was supported by grants from the Sao Paulo Research Foundation (project n. 2013/06152-7).

Young Stock

P04-004-134

Effects of milk replacer crude protein content on Holstein-Friesian and Holstein-Friesian x Jersey cross dairy heifer calf growth pre and post-weaning

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Objectives: The objective of this experiment was to compare two commercially available milk replacers with differing crude protein (CP) contents (20% compared to 26%) offered to replacement dairy heifers during the pre-weaning period on weight and average daily weight gain (ADWG).

Materials and Methods: Sixty-two replacement dairy heifer calves were enrolled on an experiment at Teagasc Moorepark Research Farm, Co. Cork from 25 January to 23 October, 2015. Calves were blocked on the basis of date of birth (DOB; 5 Feb ± 7.2 days), birth BW (33 ± 4.7 kg) and breed (42 Holstein-Friesian (HF) and 20 Jersey x HF; JEX). They were randomly assigned to one of two feeding treatments: i) fed a 20% CP milk replacer (MR20) or ii) fed a 26% CP milk replacer (MR26). Treatments were replicated. All calves were removed from their dam within one hour of birth and fed 8.5% of birth BW in colostrum in one feed. Transition milk was offered for the subsequent three days. Milk replacer was group fed, at a daily rate of 6 litres/calf, was offered twice daily for the first four weeks and once daily thereafter. All calves were offered fresh water, ad libitum concentrate and hay throughout the milk feeding period. Calves were turned out to pasture from 5 weeks of age. Calves were weaned based on a pre-defined weight; ≥ 90 kg for HF and ≥ 85 kg for JEX animals; this allowed for breed differences in mature BW. Weaning was gradual over a one-week period. Following weaning, all calves were offered ad-libitum grass outdoors. Calves were weighed weekly pre-weaning and every two weeks post-weaning. As all calves were weighed on a fixed calendar date variation existed in the actual age of calves at each weighing event. Therefore, BW ±5 days at 140 and 240 days of age is reported. Data were analysed using mixed models in SAS. The model included treatment, replicate, breed, age and birth weight.

Results: There was no interaction between treatment and breed for any of the variables investigated, thus results will not be reported. It took the MR20 calves 6 days longer to reach their target weaning weight compared to MR26 calves (93 days). As designed and imposed, actual weaning weight was the same for both treatments (94 kg). At 140 days of age the MR20 calves weighed 122 kg while the MR26 calves weighed 129 kg (P<0.05). By 240 days there was still a difference (P<0.05) between both the MR20 (205 kg) and MR26 (214 kg) treatments. Average daily weight gain from birth to weaning was 0.66 and 0.60 kg/calf/day for the MR26 and MR20 calves, respectively (P<0.01). The ADWG from weaning to 240 days old was not different between the two treatments (0.80 kg/calf/day).

Conclusions: The results from this experiment show that differences in bodyweight post-weaning are due to differences which arose during the pre-weaning period. Feeding a 26% CP milk replacer compared to a 20% CP milk replacer ensures higher ADWG during the pre-weaning period, which will increase the likelihood of heifers attaining target bodyweight at key times during their first two years of life e.g. mating start date. Furthermore, there was no interaction between breed and milk replacer suggesting that all heifers should be offered a 26% CP milk replacer compared to a 20% milk replacer.

Young Stock

P04-004-135

Immunity Development In Neonate Holstein Heifers Feeding Whole And Cell-Free Colostrum

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Objectives: The aim of this research was to evaluate the influence of colostrum cells on the development of immunity in heifers over the first 28 days of life. In this study, healthy Holstein heifers (n=19) were selected using a clinical examination between birth and two hours of life.

Materials and Methods: Heifers were distributed in two experimental groups: COL+ (n=9) which received fresh colostrum from their own dams; and group COL- (n=10) which received frozen colostrum containing no viable cells. Both groups received colostrum of the same quality measured by immunoglobulins concentration (70-120g/L – colostrometer and 23-32% - BRIX). Heifers were assessed before colostrum intake – D0; 24-48 hours after birth – D2; 7 days after birth (ab) – D7; 14 days ab – D14; 21 days ab – D21 and 28 days ab – D28. Immunological evaluations were performed using hematological assessment, immune phenotyping of, and in vitro cytokine production by peripheral blood mononuclear cells (PBMC) stimulated with BoHV-1 and bacteria. Parametric data were analyzed by unpaired T test and ANOVA for repeated measures (P<0.05), while nonparametric data was evaluated by Mann-Whitney U test and Wilcoxon test with Bonferroni correction.

Results: Peak numbers of white blood cells were observed in both groups at D7. COL- calves had a higher number of lymphocytes (Ly) (3.4 and 5.4x10³/μL) than COL+ calves (2.7 and 3.8x 10³/μL) at D0 and D7. Differences between groups in CD3⁺gamma-delta T cells were detected (10³/μL) at D0 (COL+=988.3 and COL-=1279.9; P=0.01) and D7 (COL+=1676.2 and COL-=2263.4; P=0.01). COL- calves also had more CD3⁺gamma-delta⁺ (10³/μL) at D7 (COL+=1580.6 and COL-=2352.2; P=0.00). The number of CD45⁺CD45RO⁻ (10³/μL) leukocytes was higher in COL- calves at D0 (COL+=2602.7 and COL-=3204.2; P=0.01) and D21 (COL+=951.7 and COL-=1265.6; P=0.03). Absolute values of CD45⁺CD45RO⁺ leukocytes were also different between COL+ and COL- calves at D7 (COL+=695.4 and COL-=1419.8; P=0.02). The production of cytokines by PBMC (1x10⁶ cells) was evaluated between D7 and D28 (due to low recovery of Ly at D0 and D2). PBMC, unstimulated or stimulated with BoHV-1 (Dose: 50μL, titre 10^{5.7} TCID₅₀), did not produce measurable cytokine under these conditions. PBMC from COL+calves produced more IL-17 (COL+=740pg/mL; COL-: zero) at D14 when stimulated with 5 μg of

ConA. In all calves, the PBMC response to *Escherichia coli* was higher than to inactivated *Staphylococcus aureus*[d1] . In general, PBMC from COL+ stimulated by bacteria produced IL-17 while COL- released IFN-gamma. Differences were detected between groups for interferon-gamma (COL+: zero; COL-: 225,33 pg/mL) at D21 when PBMC were stimulated with *Staphylococcus aureus*.

Conclusions: In conclusion, heifers that received whole and cell-free colostrum had a similar profile of blood Ly subsets. An increase in the number of memory cells (CD45⁺CD45RO⁺) was observed throughout the first month of life. Higher numbers of CD3⁺WC1⁻, CD3⁺WC1⁺ and CD45⁺CD45RO⁺ Ly in the COL- at D7 appeared to be associated with diarrheal disease in these calves based on clinical findings. PBMC from both groups showed greater ability to respond to bacterial stimulus than BoHV-1 under these culture conditions. However, cytokine profile presented by the two groups were different, with a higher level of Th17 produced in vitro by PBMC from COL+ calves.

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Young Stock

P04-004-136

Oral rehydration solutions containing glutamine for treating pre-weaning diarrhoea in calves : A critically appraised topic (CAT)

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Objectives: Pre-weaning diarrhoea in calves is a significant issue worldwide, with high morbidity and mortality. Oral rehydration solutions (ORS) are the mainstay of treatment in mild to moderate cases. In human medicine, the addition of glutamine, an amino acid key to providing intestinal epithelial energy, has shown varying results. Some proprietary veterinary ORS contain glutamine, and this evidence synthesis aims to evaluate the available veterinary literature to provide an evidence-based answer to the clinical question: Does inclusion of glutamine in oral rehydration solutions improve recovery from mild to moderate diarrhoea in pre-weaned calves?

Materials and Methods: Evidence-based veterinary medicine (EBVM) has been defined as using the most reliable and relevant available scientific evidence in conjunction with clinical expertise to make the most appropriate decision about a veterinary patient, while considering the circumstances of each patient and its owner (Reyher et al. 2015). The use of evidence syntheses enables the practitioner to make evidence-based clinical decisions quickly in practice, and one example of such evidence synthesis is through a critically appraised topic (CAT). To create a CAT, a clinically relevant question arising in practice is identified, and the current literature is searched and appraised in order to form an evidence-based 'clinical bottom line' with which to help base decisions (Arlt et al. 2012, Fetters et al. 2014, Steele et al. 2013). This presentation summarises a CAT produced by staff and students at the University of Bristol's School of Veterinary Sciences.

The PICO question [Population][Intervention][Comparator][Outcome] In [pre-weaned calves with mild to moderate diarrhoea] does treatment with [ORS containing glutamine] compared with [ORS not containing glutamine] lead to [improved clinical recovery]?

Search parameters (calf OR calv* OR bovi*) AND (ORT OR ORS OR oral rehydrat*) AND (scour* OR diarr*) AND glutamine

Databases searched

- Medline: 1946 - 2015 Week 46, via PubMed
- CAB Abstracts: 1973 - 2015 Week 46, using Ovid SP interface

Limits: English language only

Search performed: 24th November 2015

Results: CAB Abstracts

8 papers found: 3 papers considered irrelevant because they did not answer the question, 1 paper was inaccessible, and 4 papers were considered relevant

Medline

6 papers found: 3 papers considered irrelevant because they did not answer the question, 2 relevant papers duplicated CAB Abstract results, and 1 additional paper was considered relevant.

Total number of papers used: 5

Pal, B. & Pachauri, S. P. (2008) Effect of oral rehydration in neonatal calves treated for diarrhoea induced with *Escherichia coli* (0101:K99) infection. *Indian Journal of Veterinary Research* 17:19-26

Brooks, H. W., Hall, G. A., Wagstaff, A. J. & Michell, A. R. (1998) Detrimental effects on villus form during conventional oral rehydration therapy for diarrhoea in calves; alleviation by a nutrient oral rehydration solution containing glutamine. *Veterinary Journal* 155:263-274

Brooks, H. W., White, D. G., Wagstaff, A. J. & Michell, A. R. (1997) Evaluation of a glutamine-containing oral rehydration solution for the treatment of calf diarrhoea using an *Escherichia coli* model. *Veterinary Journal* 153:163-170

Brooks, H.W., Gleadhill, A., Wagstaff, A.J. & Michell, A.R. (1997) Fallibility of plasma urea and creatinine as indices of renal function in diarrhoeic calves treated with conventional or nutritional oral rehydration solutions. *Veterinary Journal* 154: 35-39

Nappert, G., Zello, G.A. & Naylor, J.M. (1997) Intestinal metabolism of glutamine and potential use of glutamine as a therapeutic agent in diarrheic calves. *Journal of the American Veterinary Medical Association* 211: 547-553

Conclusions: The studies varied in quality and strength of evidence, and the majority were published almost 20 years ago. Many different outcomes were measured between the studies, including villus structure, plasma volume, packed-cell volume, renal parameters, demeanour and faecal consistency. All the studies found inclusion of glutamine in a high calorie ORS was beneficial, however, where inclusion of glutamine in a non-nutritious ORS was studied, the beneficial effect was lost.

Clinical bottom line: The inclusion of glutamine in a high calorie ORS results in improved clinical recovery from diarrhoea in pre-weaned calves.

Young Stock

P04-004-137

Diagnostic accuracy of refractometry for assessing bovine colostrum quality: a systematic review and meta-analysis

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Objectives: Provision of high quality colostrum (i.e. with immunoglobulin G (IgG) concentration $\geq 50\text{g/L}$) is the first step to ensure good passive transfer immunity for young calves. Assessment of the refractive index using a Brix scale (in %) with a refractometer has been described as highly correlated with IgG concentration in colostrum. The aim of this study was to perform a systematic review on the diagnostic accuracy of the refractometry (measured on a Brix scale) to diagnose good colostrum quality (IgG $\geq 50\text{g/L}$).

Materials and Methods: Systematic review and meta-analysis of the literature were performed using CAB Abstract, Pubmed, Searchable Proceedings on Animal Conferences and Google scholar. Specific estimates of accuracy (sensitivity (Se) and specificity (Sp)) to determine good quality colostrum were obtained for the different reported cut-offs, using the cut-off of 50g/L of IgG measured with radial immunodiffusion (RID) or near infra-red spectrometry (NIRS) as the gold standard. Two by two tables (cross classification based on Brix value ($<$ or \geq Brix cut-off) and the gold standard result ($<$ or $\geq 50\text{g/L}$)) were retrieved or generated; they reported true positive, false positive, false negative and true negative cases. The prevalence of good quality colostrum was also identified or calculated. The internal and external validity of the studies were assessed using the QUALity of Diagnostic Accuracy Study tool (QUADAS2) used in human medicine. The hierarchical summary receiver operating characteristic curve model (HSROC) was used. Stochastic modeling was performed to predict the impact of meta-analysis findings on the probability of diagnosing good colostrum according to the cut-off value that was chosen and the results that were obtained.

Results: From 101 references initially obtained, 11 were included in the systematic review meta-analysis representing a total of 4251 colostrum samples. The prevalence of good quality colostrum in the selected studies varied from 67.3% to 92.3% (median 77.9%, mean 78.8%). The quality of the studies was overall good, except that the sampling strategy was not often detailed. For the Brix cut-off of 22% (n=8 studies), Se was 80.2% (95%CI: 71.1-87.0%) and Sp was 82.6% (71.4-90.0%). Decreasing the cut-off to 18% increased Se (96.1% (91.8-98.2%)) and decreased Sp (54.5 (26.9-79.6%)). Modeling of the impact of Brix accuracy showed that a positive result with the 22% Brix cut-off (ie $\geq 22\%$) can be used to diagnose good colostrum (post-test probability of a having a good colostrum: 94.3% (90.7-96.9%)). The cut-off $<18\%$ could be used to discard poor colostrum with post-test probability of good colostrum estimated at 22.7% (12.3-39.2%).

Conclusions: This systematic review revealed that there was a limited number of available studies-about Brix refractometry accuracy for colostrum quality assessment. Based on this meta-analysis of the literature, 2 cut-offs could be alternatively used to select good colostrum quality (sample with Brix $\geq 22\%$) or to discard poor quality colostrum (sample with Brix $< 18\%$). When sample results are between these 2 values, colostrum supplementation should be implemented.

Young Stock

P04-004-138

Effect of Greek oregano oil on the control of neonatal diarrhea in calves

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Objectives: Greek oregano oil yields high contents of carvacrol and thymol. These essential oils are known to possess antimicrobial, antiparasitic, antifungal and antioxidant activities. Based on these properties, oregano oil has been successfully used for controlling diarrhea syndrome in animal species other than calves. Neonatal diarrhea is considered as the most common syndrome occurring in calves under 15 days old and an important cause of financial losses in dairy herds. The objective of this study was to evaluate whether the daily administration of Greek oregano oil is effective in diminishing the severity of calves' neonatal diarrhea syndrome.

Materials and Methods: The study was conducted in a dairy farm with high incidence rate ($>90\%$) of diarrhea syndrome in calves aged less than 10 days old, mainly caused by Rotavirus. The calves of 30 cows vaccinated against Rotavirus, Coronavirus and Enterotoxigenic E. coli that were expected to calve within two months were included in the study. The calves alternately assigned into two groups based on the birth date. The first group (E) consisted of 15 calves that were receiving once daily 12.5 mg/kg Greek oregano oil (Ecodiard liquid 5%) for the first 10 days of their life and the second one (C) of 15 calves that received no treatment and served as controls. After calving, calves of both groups were fed 2 lt of colostrum within 2h. Soon after, the calves of group E were additionally drenched with the respective amount of oregano oil. The drenching was being repeated daily after the morning feeding. During the study period of 15 days, faeces were scored every morning using the following scale: 1=normal, 2=pasty, 3=watery. Fecal samples were obtained from the rectum on days 3, 6 and 10 and on the first day of diarrhea (fecal score, FS ≥ 2) for Cryptosporidium spp. oocysts counts and bacteriological examination. A commercial snap test was also performed with the diarrheic faeces to detect viral agents. The diarrheic calves of both groups were receiving the routine farm treatment. Based on the records, the number of days until recovery (FS=1) were obtained and the average diarrhea fecal score and diarrhea index (FS x days with diarrhea) were calculated. The data were analyzed with SPSS® version 22.

Results: Diarrhea was observed in 27 out of 30 calves used in the experiment. The incidence rate of diarrhea syndrome was unaffected by oregano oil (13/15 and 15/15 for groups E and C, respectively; $P>0.05$). Rotavirus was identified as the causative agent of diarrhea in 11 out of 12 diarrhea cases in group E and in 12 out of 15 cases in group C. The average faecal score throughout the experiment was significantly lower in group E than group C (1.24 ± 0.063 and 1.52 ± 0.073 for groups E and C, respectively; $P<0.05$) and on days 8 to 12 of the study ($P<0.05$). The average duration of diarrhea was significantly lower in group E compared to group C (3.08 ± 0.26 days and 5.13 ± 0.68 days for groups E and C, respectively; $P<0.05$) as well as the diarrhea index (7.65 ± 0.68 and 13.54 ± 1.80 for groups E and C, respectively; $P<0.05$). The average diarrhea faecal score was not significantly different between groups (2.48 ± 0.09 and 2.60 ± 0.08 for groups E and C; $P>0.05$). Cryptosporidium spp. oocysts were sporadically identified in low burdens in the faecal smears of the calves.

Conclusions: The administration of Greek oregano oil, on a daily basis, for the first 10 days of the calves' life appeared to be effective in reducing the severity of neonatal diarrhea syndrome caused by Rotavirus.

Young Stock

P04-004-139

Study of Haptoglobin Kinetic in Holstein Heifers from birth up to 10 Months of Life

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Objectives: Haptoglobin is an important inflammation biomarker for bovine and can be used in screening for diseases that affect heifers. Thus, the objective of this research was to study the kinetic of haptoglobin (Hp) in healthy Holstein heifers between the first day up to the tenth month of life.

Materials and Methods: For this, 363 Holstein heifers were examined, among which 244 healthy animals were selected. Animals were distributed according to age bracket and divided in 10 groups: 0 - 30 days (M1, n=16), 31 - 60 days (M2, n=15), 61 - 90 days (M3, n=32), 91 - 120 days (M4, n=14), 121 - 150 days (M5, n=35), 151 - 180 days (M6, n=25), 181 - 210 days (M7, n=58), 211 - 240 days (M8, n=12), 241 - 270 days (M9, n=24) and 271 - 300 days (M10, n=13). Blood serum samples were used to determine the concentration of Hp based on the capacity of ligation to meta-hemoglobin molecules, using a control serum with known concentrations of Hp and standard curve prepared in serial dilution. Guaiacol was used as substrate for the reaction. The determination of serum haptoglobin concentration was calculated by linear regression after spectrophotometric determination of absorbance in microplate reader and wavelength 450 nm. Data did not present parametric distribution, thus, medians between moments were compared by Dunn's test. Difference between groups were considered significant when $P < 0.05$.

Results: Medians, minimum and maximum values of haptoglobin (mg/dL) for the different groups were: M1 = 2.45 (1.01 - 5.30); M2 = 1.69 (0.76 - 9.91); M3 = 1.51 (0.64 - 5.60); M4 = 1.58 (0.00 - 9.56); M5 = 3.13 (0.83 - 5.52); M6 = 3.59 (2.50 - 6.11); M7 = 3.78 (0.00 - 5.85); M8 = 3.98 (2.63 - 6.28); M9 = 3.96 (2.54 - 6.44); M10 = 3.85 (2.32 - 5.95). After statistical analysis, it was possible to verify significant difference between M3 group in relation to groups M5 to M10. M4 also presented significant difference when compared to M6, M7 and M9.

Conclusions: It was possible to determine reference ranges and influence of age for Hp in dairy heifers in the first 10 months of life. This is a significant finding for the correct interpretation of the presented values and differentiation of healthy and sick animals.

Young Stock

P04-004-140

Effect of Saccharomyces cerevisiae and mannaoligosaccharides on average daily gain and health in Holstein calves under Chilean conditions

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Objectives: Saccharomyces cerevisiae and its cell wall (glucan and mannan) have been used as an additive in young cattle improving calf growth and performance. These products have not been studied under Chilean conditions; consequently, the objective of this study was to compare the effect of S. cerevisiae, and a β -glucan-mannan based product on average daily gain (ADG) and health of young calves under Chilean conditions.

Materials and Methods: The study was conducted on a dairy farm from central Chile. The farm milked 1,100 cows, producing. At birth, calves were immediately removed from the dam, and fed colostrum according to a standard protocol. Calves were placed in an individual hutch bedded with straw. In order to find a difference of 35 gr on ADG between treated and control groups, with a 95% of confidence and 80% of power, a sample size of 25 calves per group was calculated. A blood sample was obtained between 2 and 8 d of age and serum total protein was measured to monitor passive immune transfer. Calves were fed a milk replacer (21% crude protein, 17% fat) at a concentration of 14.3%, 2 liters, twice a day until 15 days old, and then, 3 liters twice a day until weaning. Starter was pelleted (21% crude protein, 2.5% fat, 11% crude fiber) and was offered with water free choice from 3 days old until weaning. Experimental groups were: G1 = no additives; G2= 3 g/calf/day of mannaoligosaccharides diluted in the milk replacer during the morning feeding (Safmannan®, Lesaffre, France) and G3= 5 g/calf/day of a live yeast culture of Saccharomyces cerevisiae (10×10^9 live cells) diluted in the milk replacer during the morning feeding (Procreatin-7®, Lesaffre, France). Calves were weighed on a scale at 0, 30 and 60 days old before feeding and ADG was calculated. During the study diarrhea and pneumonia events were recorded. ADG was analyzed by ANOVA. Explanatory variables were the treatment, parity of the dam, and calf serum total protein. Incidence of diseases was analyzed through a logistic regression model.

Results: Weaning was carried out at 60 days of life. Body weight at weaning was 71.6 ± 2.5 kg^a, 74.6 ± 2.2 kg^{ab} and 75.9 ± 2.7 kg^b for G1, G2 and G3, respectively ($P < 0.05$). Average daily gain between birth and weaning were 0.56 ± 0.1 kg^a; 0.61 ± 0.11 kg^{ab}; and 0.63 ± 0.1 kg^b for G1, G2 and G3, respectively ($P < 0.05$). Incidences for diarrhea and respiratory diseases in conjunction were 32.3%, 32.4%, and 20.5%, for G1, G2 and G3, respectively. These differences were not statistically significant ($P = 0.21$).

Conclusions: Live yeast culture of Saccharomyces cerevisiae improved significantly average daily gain and tended to decreased diseases occurrences in dairy calves between 0 to 60 days of life managed under Chilean conditions.

Young Stock

P04-004-141

Associations of serum amyloid A and haptoglobin with lower weight gain, group size and respiratory disease in dairy calves

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Objectives: The aim of the study was to explore the associations between the acute phase proteins (serum amyloid A and haptoglobin),

group size, respiratory diseases and growth of young dairy calves in calf rearing unit.

Materials and Methods: The study consisted of 480 calves gathered in a calf rearing unit in Northern Finland. A lot of 80 calves, aged 10-35 days, was gathered weekly to the rearing unit. In the trial calves were randomly allocated to control group (LARGE = 40 calves per pen) or experimental group (SMALL = 10 calves per pen, 4 pens) on arrival. The study was repeated for six lots.

SMALL groups were separated from each other and from the LARGE group with solid fences preventing direct contact between calves in different pens. Groups (1 LARGE and 4 SMALL) stayed in one compartment for 80 calves to ensure similar environmental conditions, and shared the air space.

The calves in LARGE and SMALL groups were clinically examined for respiratory diseases, umbilical diseases and diarrhea on arrival, and at 3 and 6 weeks from arrival. Blood samples for haptoglobin, serum amyloid A (SAA) and serology (respiratory syncytial virus, parainfluenza 3 virus, bovine adenovirus and Mycoplasma bovis) were obtained at all clinical examinations. Calves were weighed on arrival to rearing unit and when moved to another compartment after the initial rearing period of 49 ± 5 days, 0-16 days after the third examination.

The workers of the farm followed the calves between the clinical examinations. Calves with respiratory tract disease received antimicrobial treatment if they had body temperature ≥ 40.0 °C or respiratory rate > 60 /min.

Associations between the variables were explored using linear mixed models, lot and pen inserted as random factors. The model was also adjusted for repeated measurements.

Results: Increased serum amyloid A concentrations in calves at the beginning of rearing period (first sampling time) were associated with lower weight gain during rearing period (approximately 50 days). Calves treated for respiratory diseases at the third sampling time had higher SAA and Hp concentrations at the same sampling. Calves from smaller group size had higher SAA concentrations (SMALL 53.0 ± 36.6 mg/l, LARGE 49.2 ± 388.3 mg/l) and higher Hp concentrations (SMALL 285.8 ± 301.7 mg/l, LARGE 264.5 ± 281.5 mg/l) at the end of rearing period (=third sampling time).

Conclusions: The study detected an association between increased SAA concentration and lower weight gain on following weeks, similarly to our earlier results in beef calves (Seppä-Lassila et al, submitted) and reindeer calves (Orro et al 2006). The mechanisms for this phenomenon are yet to discover, but it seems that events affecting immature immune system can have long lasting effects.

Acute phase proteins are seen as markers of inflammation and stress. The general consensus exists that smaller groups would benefit calf health and create a less stressful environment. The results concerning group size in the current study warrant for further studies.

Young Stock

P04-004-142

Time of milk replacer introduction: its effects on pre and post weaning growth.

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Objectives: Early introduction of milk replacer (MR) feeding could be a future strategy used to reduce transmission of Johnes Diseases to neonatal calves. The aim of this experiment was to investigate the effect of the timing of introducing MR into the diet of dairy heifer calves on body weight (BW) during the pre- and post-weaning periods.

Materials and Methods: The experiment was carried out at Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co.Cork, Ireland. A study population of 120 dairy heifer calves (Holstein Friesian and Holstein Friesian x Jersey) was used. Calves were weighed immediately after birth (mean BW at birth 33.2 (S.D 3.34) kg) and received 8.5 % of their birth BW in colostrum, obtained from the first milking of the dam within two hours of birth, and was fed via oesophageal tube. Calves were then randomly assigned to one of three treatment groups: (i) fed milk replacer at the feed following colostrum feeding (MR), (ii) fed milk replacer following colostrum feeding and four feeds of transition milk (TM) and (iii) fed milk replacer following colostrum feeding, four feeds of transition milk and ten feeds of whole milk (WM). Milk replacer (26 % crude protein) was fed at a rate of 15 % of birth BW and prepared at 15 % concentration. Weight recording was carried out weekly from birth to weaning to monitor the effect of the treatments on BW. Calves were weaned when they reached 18 % of mature BW and the age at weaning was recorded. Post-weaning, weight was recorded at two week intervals.

Results: There was no difference in BW at birth (33.2 kg) or at weaning (93.2 kg) between the three treatments. There was also no difference in the time taken to reach target weaning weight (90.2 days), and consequently no difference in average daily gain (ADG) from birth to weaning (0.66 kg/day) between treatments. When calves were weighed at 140 and 240 days old again there was no difference in BW (129 and 215 kg, respectively). Similarly there was no difference in ADG from birth to 240 days old (0.75 kg/day) and weaning to 240 days old (0.81 kg/day) between treatments.

Conclusions: Early introduction of MR feeding did not affect BW or ADG of dairy heifer calves in the pre- and post-weaning period. This suggests that milk replacer can be offered to calves immediately after colostrum feeding where there is a risk of Johnes disease transmission to neonatal calves, however colostrum feeding needs to adhere to best practice.

Young Stock

P04-004-143

Changes in neonatal beef calf blood chemistry during the first 72 hours of life

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Objectives: The objective of Experiment 1 was to investigate the effect of age on serum chemistry panel measures in suckling neonatal beef calves during the first 72 hours. The objective of Experiment 2 was to determine the effect of maternal forage system during late gestation on neonatal calf serum chemistry.

Materials and Methods: In Experiment 1, a total of 66 fall-calving beef cows and heifers were monitored during calving, and calf blood samples were obtained from a subset ($n = 24$; average dam age = 4.4 ± 0.5 years; average dam BCS = 5.2 ± 0.1 ; average calving date = September 11, 2015). Jugular blood samples were obtained from 8 bull and 16 heifer calves at 0, 6, 12, 24, 48, and 72 hours postnatally for determination of serum chemistry profile. Samples at 0 hour were obtained prior to colostrum intake but after standing. Cow-calf pairs were managed together in large pens, and calves were allowed to suckle normally. Data were analyzed using sampling hour as a fixed effect, and least square means were compared to adult bovine reference ranges used by the University of Missouri Veterinary Medicine Diagnostic Laboratory. In Experiment 2, 56 multiparous, spring-calving crossbred beef cows either strip-grazed stockpiled growth of tall fescue (High quality forage; 12.4% crude protein, 64.1% in vitro total digestibility, dry matter basis; $n = 4$ pastures) or consumed summer-baled tall fescue hay (Low quality forage; 7.6% crude protein, 57.7% in vitro total digestibility, dry matter basis; $n = 4$ uncovered drylots) beginning on day 188 ± 2 of gestation. At 52 ± 0.6 hours of age, calf jugular blood samples were obtained for determination of serum chemistry profile. Data were analyzed using forage system as a fixed effect; calf date of birth and sex were included in the model when $P < 0.25$. Pasture or drylot was the experimental unit. $P \leq 0.05$ was considered to be significant in both experiments.

Results: In Experiment 1, all serum chemistry measures except bicarbonate were affected by sampling hour ($P \leq 0.001$). Serum glucose, urea nitrogen, total protein, and globulin concentrations of neonatal beef calves increased from 0 to 24 hours, then leveled. Concentrations of magnesium, total and direct bilirubin, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), and creatine kinase (CK) increased during the first 12 hours, then decreased from 24 to 72 hours of age. Serum creatinine decreased from 0 to 72 hours, whereas serum albumin, calcium, and anion gap decreased during the first 24 hours. Phosphorus and potassium concentrations decreased from 0 to 6 hours, but increased from 24 to 48 hours. Serum sodium and chloride had only slight changes from 0 to 72 hours of age. At some sampling hours, mean glucose, albumin, total protein, globulin, AST, and CK were below their reference range. Mean glucose, creatinine, potassium, chloride, calcium, phosphorus, magnesium, anion gap, total bilirubin, direct bilirubin, GGT, and CK were also above their references range at specific sampling hours. Urea nitrogen and sodium were within their reference ranges for all sampling hours. In Experiment 2, calf birth weight tended to be 10% less for calves born to cows fed low quality forage during late gestation ($P = 0.08$), indicating reduced fetal growth. Calves born to cows fed high quality forage had increased serum creatinine ($P = 0.05$) and albumin ($P = 0.04$) at 52 hours of age; other neonatal serum chemistry measures were not affected by forage system ($P > 0.05$).

Conclusions: Serum chemistry profiles of suckling, neonatal beef calves are impacted by sampling time, which should be taken into consideration when evaluating individual animals. Several measures also may be outside of adult bovine normal references ranges used in many clinical settings, suggesting that neonatal calves need specific reference ranges based on age. Additionally, maternal nutrient intake during late gestation may affect serum chemistry of neonatal calves, even at 52 hours of age.

Young Stock

P04-004-144

Intestinal villi integrity with supplementation of *Saccharomyces cerevisiae* fermentation products in neonate calves with cryptosporidiosis

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Objectives: The main objective of the study was to characterize the integrity of intestinal villi in neonatal calves naturally infected with *Cryptosporidium* spp. subjected to a daily supplement of *Saccharomyces cerevisiae* fermentation product for 28 days. This study is part of a larger investigation on oocyst shedding and quantitation in dairy female and bull calves treated with the described products.

Materials and Methods: The study took place in a dairy of 1, 100 cows in production in Querétaro, México. 15 dairy bull calves were removed from the calving pens and randomly assigned to their individual hutches receiving one of three treatments: *Saccharomyces cerevisiae* fermentation products (T1); a commercial supplement with mananoligosaccharides (MOS) and yeast (T2); and maltodextrin as control. Treatments were delivered in colostrum, milk and pre-starter for 28 days. At the end of the neonate period all calves were humanly sacrificed at the University premises. Samples from rumen, abomasum, duodenum, jejunum and ileum were taken for histopathology analysis. Slides were stained with hematoxylin-eosin (HE) and measurements were performed in a Carl Zeiss microscope with the Zen system, reading and recording images at 40 X (10 fields per calf). Intestinal villi were classified to assess integrity in total number, normal, disrupted, atrophied and blunt. Intestinal height, width and crypt size were also measured. Sampling for *Cryptosporidium* spp. oocysts took place at 0, 7, 10, 14 and 28 day old calves, quantified with a modified Sheather's technique by Arrowood. Statistical analysis was performed on JMP 11.0.0 (license N15DC1JJ0R). For height, width and crypt size, as well as for intestinal villi integrity with parametric analysis such as Hsu's MCB (multiple comparisons for Best) and Wilcoxon test for non-parametric analysis.

Results: The number of villi in each field varied from 10.1 to 12.5 per campus. When comparing the total number of villi for field groups T1 and T2 for ileum were different to C (p -value < 0.05). Regarding duodenum, scores were similar for all treatments. In case of jejunum, disrupted villi were less in case of treatments T1 compared to T2 and C (p -value < 0.05); atrophied villi were more in T2 and C when compared with T1 (p -value < 0.05); T1 had more normal villi when compared to T2 (p -value < 0.05) and control (p -value < 0.0002). In the case of ileum, total villi were more for T1 (p -value < 0.05); T2 showed more for blunt villi (p -value < 0.02); regarding normal villi T1 showed more than T2 (p -value < 0.0008) and with C (p -value < 0.02). In case of width, height and crypts of intestinal villi the main differences were: in duodenum T1 were wider than T2 and C (p -value > 0.02) while T1 were higher than T2 and C (p -value < 0.0001) with Hsu's MCB and Wilcoxon tests. For jejunum T1 and C were wider than T2 (p -value < 0.0001) with Hsu's MCB and Wilcoxon tests. Crypts in jejunum were T2 different to T1 and C (p -value < 0.0001) with Hsu's MCB and Wilcoxon tests. For ileum T1 and T2 were different from C in height (p -value < 0.0001) with Hsu's MCB and Wilcoxon tests and width (p -value < 0.01). 100% of the bull calves showed *Cryptosporidium* spp. oocysts from 80×10^3 to $4,040 \times 10^6$ in at least two of the five samplings.

Conclusions: Treatment with the *Saccharomyces cerevisiae* fermentation product showed higher numbers of total intestinal villi per field, as well as more normal villi in case of jejunum and ileum. Duodenum, jejunum and ileum villi were wider and higher with the studied treatment. Intestinal villi integrity, and particularly ileum, where it is reported that *Cryptosporidium* spp. causes severe damage, regardless of the prevalence and number of oocysts, was better in case of the bull calves treated with *Saccharomyces cerevisiae* fermentation. The product shows promising results as a villi defender against *Cryptosporidium* spp.

Comments: Commercial names were taken out of the document T1 is Smartcare and Diamond V XPC, T2 is Biomox and T2 maltodextrin.

Young Stock

P04-004-145

Associations between average daily gain and calf health, feeding, and management practices in preweaned dairy heifer calves in the U.S.

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Objectives: Preweaned dairy calves are traditionally fed lower amounts of milk compared to their beef counterparts. Feeding less milk or milk replacer is associated with lower average daily gains (ADG) and may also lead to increased morbidity and mortality. The objective of this study was to evaluate associations between average daily gain and calf health, feeding, and management practices in preweaned dairy heifer calves in the U.S.

Materials and Methods: This longitudinal study was conducted as part of the National Animal Health Monitoring System's (NAHMS) Dairy 2014 study from March 2014 through August 2015. This study focused on dairy heifer calf health and management from birth to weaning. During the preweaning period for enrolled calves, all health events were recorded and calf growth was assessed at 2-week intervals. Liquid diets were categorized by type (i.e., milk replacer, waste/whole milk, or a combination) and by volume (i.e., > 5.8 kg/day or ≤ 5.8 kg/day). Proc Mixed in SAS was used to determine which diet, health and management practices were significantly associated with ADG ($p < 0.05$). This interim analysis is based on 1,541 calves from 97 operations (approximately 60% of the expected total). A network analysis will be conducted to determine the most important factors impacting ADG, and results will be presented.

Results: A total of 104 dairy operations in 13 states participated, with an average of 24 calves enrolled per operation. The average ADG was 0.7 kg/day (range: 0.08-1.89 kg/day). The average age at weaning was 62.6 days or 8.9 weeks (range: 31-193 days). Calves, on average, gained 47.7 kg during the preweaning period. The average amount of liquid fed per day was 5.8 kg. Whole/waste milk was fed to 38.0% of calves, milk replacer to 36.1%, and a combination of the two was fed to 25.9% of calves. The preliminary model estimating ADG included disease event, liquid diet, season, and the interaction between diet and season. Calves without disease had a higher ADG than calves with one or more disease event. There was a complex interaction between diet, season, and ADG. Further analysis will be conducted to help explain the relationship.

Conclusions: Preliminary results show improved ADG in calves not experiencing a morbidity event. The relationship between ADG, season, and liquid diet will be further evaluated with a complete dataset. Growth in preweaned heifer calves is complex and affected by multiple facets of calf management, which can be modified to improve calf growth and reduce disease occurrence. Improving growth through reduction of disease occurrence can improve animal welfare and decrease the need for antibiotics.

Young Stock

P04-004-148

Glossitis indurativa diffusa et chronica caused by the pasture of bamboo

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Objectives: The mouth and especial the tongue is for the differential diagnosis of several severe infectious diseases of special importance. Several deaths in post weaning young stock beef cattle were observed after periods inanition and ingestion of bamboo. The objective is to report a case of chronic traumatic glossitis in a heifer.

Materials and Methods: A 10 months old crossbred (Angus x Nelore) heifer was attended at the university Hospital (UHAF/PUCPR - Pontifícia Universidade Católica do Paraná) belonging to an extensive raising beef cattle farm in the county of Fazenda Rio Grande - Paraná State. On bad managed pasture causing hunger several animals were reported grazing nearby native bamboo. Reported inanition in the winter months caused deaths in former years. Ingestion of bamboo and similar symptoms as the reported case were observed. The farm was inspected and the native bamboo called "taquara or cara" was identified belonging to the genus *Merostachys* spp.

Results: The heifer was chronically underdeveloped, cachectic weighing 124,5 kg with disproportional development of the head and body. The hair was dull, the rumen empty and the mandibular lymph nodes slightly increased. The inspection of the oral cavity revealed several scars at the tip of the tongue, that assumed a small balloon like aspect, with the thicker end at the tip. The tongue was firm and painful at palpation, with nearly no mobility. At the proximal lateral region of both sides of the tongue fresh small ulcers were noted, caused by sharp teeth points due to irregular abrasion of the molars. The heifer had extreme difficulty to apprehend the food (TMR) offered. Other clinical parameters were in the normal range. Diagnosis was a diffuse chronic glossitis caused by the ingestion of bamboo and infection of ubiquitous oral bacteria causing cachexy. Treatment was instituted with correction of the teeth followed by 1 oral application of gentiana violet. Glossitis treatment was instituted with phenylbutazone (9 mg/kg IV/SID once and 4,5 mg/kg IV SID-4 days), high dosage of penicilline (35000 IU/kg for 15 days associated with IV-Lugol(Götze)solution - 3 applications in intervals of 1 week. After 3 days of treatment ingestion of ration improved and after 5 days swelling of the tongue decreased allowing better feeding behaviour. At the end of the treatment the tongue improved mobility and consistency. Then the heifers weight was 149,5 kg. For observance of possible recidivance and education purpose the animal was maintained for another month and left the hospital weighing 196 kg.

Conclusions: To our knowledge this is the first report of famine with consequent ingestion of bamboo (*Merostachys* spp.) causing chronic glossitis. Other tropical areas where different bamboo species occur might be alerted that the very splintery material could cause severe lesions to the oral cavity. Complete clinical reversion of chronic indurative glossitis with the instituted treatment was not possible, but survival was guaranteed.



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Young Stock

P04-004-149

An evaluation of pathogens and risk factors associated with neonatal enteritis in calves

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Objectives: The study had two objectives:

1) An evaluation of the cumulative incidence of neonatal enteritis (based on farmer treatment records), associated cumulative mortality (based on British Cattle movement Services (BCMS) data) and pathogens associated with neonatal enteritis on a subset of practice farms.

2) An examination of risk factors associated with the cumulative incidence of neonatal enteritis and its associated cumulative mortality on a subset of practice farms.

Materials and Methods: 47 dairy and beef farms, with a UK wide distribution and from a single Veterinary practice contributed data to the study.

Information on calf management practices and client perception of the incidence of neonatal enteritis and mortality within the first three weeks of life, were gathered in a quantitative fashion, through the development and distribution of a questionnaire (Boynton and Greenhalgh 2004).

Client questionnaires were not anonymous, with all respondents identified by both personal and farm names. This allowed correlation of client perception of the incidence of neonatal enteritis and neonatal calf mortality within the first three weeks of life to be paired against actual disease incidence and mortality rates on farm.

Surveillance for enteric pathogens was undertaken when a diagnosis of neonatal enteritis was made by the attending veterinarian, either during routine calf health monitoring or sick calf visits, utilizing the validated "Cow-side ELISA" Scour Check Rapid™ (MSD Animal Health), to test for a fixed group of major pathogens associated with neonatal enteritis (Rotavirus, Coronavirus, E.coli F5 (K99), Cryptosporidium parvum).

Data from returned questionnaires was collated into the study database (Microsoft Office Excel, 2007 Microsoft Corp) and statistical analysis performed using Epi-Info version 3.5.4 (CDC) statistical software.

Results: Data analysis revealed a mean neonatal enteritis cumulative incidence of 26% (95% CI=18.11 to 33.89) across the studied farms, determined from farmer treatment records and the mean cumulative mortality to be 4.5% (95% CI= 2.65 to 6.35) as determined from BCMS data.

Rotavirus was the most prevalent pathogen found, with 70% of calves tested showing exposure (n=35). Cryptosporidium parvum was the second most common pathogen accounting for 40% (n=20) of infections. Of the calves sampled, 40% had combined Rotavirus and Cyptosporidium parvum infections (n=20).

Analysis of BCMS data revealed a significant association in terms of higher neonatal calf cumulative mortality in the first twenty one days of life in those herds relying on self feeding from dam, compared to those actively administering colostrum to newborn animals (RR= 2.83, 95% CI= 1.01 to 7.91, p=0.045 Fishers Exact test).

Analysis of BCMS data showed a significant association between those herds utilizing dedicated youngstock staffing and a reduced neonatal calf cumulative mortality within the first twenty one days of life (RR= 2.5, 95% CI= 1.25 to 5.01, p=0.001 Fishers Exact test).

Analysis of BCMS data showed a significant association between the presence of circulating BVD virus and an increased neonatal calf

cumulative mortality within the first twenty one days of life (RR= 0.30, 95% CI= 0.08 to 1.13, p=0.04 Fishers Exact Test).

Farmer perception bore NO relationship to the actual cumulative incidence of neonatal enteritis experienced on farm.

Conclusions: Within this study the reliance upon self-feeding from the dam as the sole means of passive transfer, the employment of dedicated youngstock staff and the presence of circulating BVDV were all found to have a significant association with neonatal calf cumulative mortality in the first twenty one days of life.

These factors compounded by the finding of farmer perception bearing NO relationship to the actual cumulative incidence of neonatal enteritis experienced on farm may warrant further investigation using a larger dataset and may serve as factors for consideration in future youngstock health audits and knowledge transfer programs.

Young Stock

P04-004-150

Blood transfusion in young calves in case of illness

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Objectives: Blood transfusion in cases of weak calves is something so beneficial that we can choose to describe it as good, very good or even... spectacular! Since there is little said about that topic in literature, we decided to sum up our experience.

Materials and Methods: We often find calves in their first days or weeks of life which are very weak, either from birth, or following some kind of illness. Sometimes calves lack colostrum, making them much more susceptible to disease. But even if they are fed the best colostrum they may still end up septic, causing them to dehydrate rapidly as they have very little body reserves. Diarrhoea and pneumonia, caused by viruses or bacteria, appear constantly among young calves.

We have also cases where calves remain weak despite therapy. Anaemia coupled with an insufficient immune response brings the whole organism close to death again. In such a situation we attempt blood transfusion. With it we support the calf's natural defense systems in combat against intruders (viruses, bacteria). In cases where we have a certain degree of dehydration, we also maintain a better fluid supply. If a calf needs a transfusion, it needs a transfusion – or it will die. So it is worth a try. All we need is a collecting bag, commercially available. For example, human disposable bags, which can collect up to 450 ml. In such cases (more often in dairy herds, where we have abandoned calves, which value very little in money), long or prolonged treatment is not acceptable for the farmer. That is a sad fact, but we can do little against it. The drug industry doesn't support research in this field because they have no interest in these procedures. Due to the fact that the "drug" is not something to sell, but is just solid, crude tissue, real blood, taken out of the body on site. So far, it's for free.

Results: The donor cow should preferably be its mother. If the mother is not available, we can use another cow from the herd. We have not had bad experiences with anaphylaxis, even if the treatment was repeated twice. The main focus is adding more antibodies from a healthy cow directly to the calf. To take blood from the cow we need blood bag. The amount of blood in one bag is appropriate for a young calf weighing from 40 to 100 kg. After administrating the needle into the abdominal vein (in dairy cattle this is not difficult) it takes about 10 minutes to collect a full bag. Gently agitate

the collected blood to mix it with the anticoagulant solution. Once the bag is full, run a small amount out of the line onto the ground to prevent blood clotting in the line or needle. The calf is then restrained (often it is too weak to fight back). The area of the jugular vein is clipped and an IV catheter or needle is placed. We then attach the blood bag to the needle and give the calf blood. A bag could be emptied in 10 to 15 minutes without risk to the recipient. Within several hours the calf will often stand up and start drinking on its own. It's rare that a calf needs a second transfusion. It can drastically help the organism if it is done early enough in the disease process. Another benefit of this type of procedure is that the blood is always at the proper temperature for transfusion since only a few minutes pass between taking the blood from the donor and giving it to the calf. The benefit of this is not to be ignored, as many calves that require a transfusion are already hypothermic.

Conclusions: Blood transfusion can be done in field conditions easily and is performed as an emergency life-saving procedure. It costs very little, especially in comparison with the excellent benefits it brings. It is worth doing from a welfare and treatment aspect. The procedure is also very satisfying for veterinarians and farmer clients. In our veterinary practice, VPB, we started years ago in cases where nothing else could be done. This "ultima ratio" experiment gave us an example of good and successful practice with a herd of saved cases. Give it a try!

Young Stock
P04-004-151

Bacterial count in frozen spare supply of colostrum on 42 dairy farms around Someren (Netherlands)

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Objectives: The bacterial count is besides the concentration of antibodies, most specifically IgG, the most important component of colostrum quality (Stewart et al., 2005; Godden, 2008). An increased bacterial count has a negative effect on the absorption of IgG in the intestinal tract of the newborn calf (Langel et al., 2015). The upper limit of bacterial counts in milk and colostrum is generally defined as 100.000 cfu/ml (i.e. Morrill et al., 2012).

The goal of this study was to analyze the bacterial count and coliform count of frozen spare supply of colostrum on 42 dairy farms, all clients of Veterinary Center Someren.

Materials and Methods: On 42 dairy farms that were clients of Veterinary Centre Someren, 95 samples were aseptically taken from frozen colostrum that serves as spare supply to be fed as a first meal to newborn calves when there is no colostrum from the own dam available. At the moment the farmers put the colostrum in the freezer, they did not know about this study.

The samples were transported in cool boxes and arrived in frozen condition at the laboratory of VC Someren, that performed bacterial counts. The results of 11 samples were not used because these samples were not representative: the farmers had handled and frozen this colostrum in a different way than they normally would have done.

In 64 of the samples also the coliform count was analyzed. In the first batch of 20 samples this test was not performed.

Results: 58% (49 of 84) samples had a bacterial count higher than 100.000 cfu/ml and 10% had a bacterial count higher than 100.000.000 cfu/ml.

17% (12 of 64) samples had a coliform count higher than 10.000 cfu/ml.

Dairy farmers in this area keep a spare supply of frozen colostrum to feed to newborn calves in cases that there is no or not enough fresh colostrum available. This colostrum is mosttimes used in cases there is no or not enough fresh colostrum available for a newborn calf.

It is assumable that the bacterial count will further increase during the process of thawing, handling and feeding this colostrum.

Conclusions: 58% of the 84 sampled portions of refrigerated colostrum stored on 42 dairy farms around Someren (NL) had a bacterial count higher than the internationally maximum standard of 100.000 cfu/ml.

17% of 62 sampled portions had a coliform count higher than the international maximum standard of 10.000 cfu/ml.

Comments: The process of selection plus the number of dairy farms make it highly assumable that the results are a reliable reflection of the situation amongst all the clients of this veterinary practice. There are no reasons to assume that this group of dairy farmers is not representative for the whole dairy farmers population in at least the south of the Netherlands.

Young Stock
P04-004-152

Bacterial count in the first meal of colostrum at the moment of feeding on 17 dairy farms around Someren (Netherlands)

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Objectives: The bacterial count is besides the concentration of antibodies, most specifically IgG, an important component of colostrum quality (Stewart et al., 2005; Godden, 2008). An increased bacterial count has a negative effect on the absorption of IgG in the intestinal tract of the newborn calf (Langel et al., 2015). The upper limit of bacterial counts in milk and colostrum is generally defined as 100.000 cfu/ml (i.e. Morrill et al., 2012).

The goal of this study was to analyze the bacterial and coliform count of colostrum at the moment it is fed as first, second or third meal to newborn calves dairy farms, all clients of Veterinary Center Someren.

Materials and Methods: Samples were taken by dairy farmers, who had received sampling instructions on a chart and a sample kit.

A normal operating procedure after calving is to milk out the dam completely and feed the first colostrum to the calves in separate meals, until it is finished. Then the calve receives meals with colostrum from a second or later milkings.

The sample kit consisted of 5 sterile 5 ml disposable syringes with a sterile 1.2 mm x 3.5 mm (pink) needle, plus 10 milkers gloves. Instruction was to aspirate colostrum into the syringe via the needle and immediately put the syringe plus covered needle into a refrigerator. During the sampling period the farmers were aware of the results of an earlier studie of the bacterial count of frozen colostrum supply on dairy

farms, in which 58% of the samples exceeded the maximum bacterial count of 100.000 cfu/ml.

The samples were collected, transported in a cool box and arrived in frozen condition at the laboratory of VC Someren, that performed bacterial counts. On 17 dairy farms 86 samples were taken and analysed. 6 samples were not used in the analysis, because they were not taken from first colostrum or the colostrum was not handled in the normal way by the farmer.

Results: 24% (17 of 70) of the samples of the first colostrum meal had a bacterial count higher than 100.000 cfu/ml and 7% (5 of 70) had a coliform count higher than 10.000 cfu/ml.

30% (3 of 10) of the samples of the second colostrum meal had a bacterial count higher than 100.000 cfu/ml and 10% (1 of 10) had a coliform count higher than 10.000 cfu/ml.

The number of samples of second colostrum meals is too low for significant conclusions. It can be assumed that the bacterial count and coliform count will increase over time in many cases, as very few farms were quickly refrigerating the remaining part of the colostrum.

Conclusions: 24% (17 of 70) of the samples of the first colostrum meal had a bacterial count higher than 100.000 cfu/ml and 7% (5 of 70) had a coliform count higher than 10.000 cfu/ml.

In practice, a substantial part of colostrum that is given as a first meal to newborn calves has a too high bacterial count.

Comments: Most of the practical work in this study was performed by Jens Verheijen.

for the WBC abstract committee: In the Dutch magazine Tijdschrift voor Diergeneeskunde of the Royal Dutch Veterinary Society KNMvD, Jan Hulsen published an article on colostrum that also mentions this study. This article is in Dutch and has no English summary or what so ever. Therefore the authors think it is relevant that this study as such will be published, hopefully at the WBC. To share the info with the whole world and not just with the Dutch veterinarians.

Young Stock

P04-004-153

Use of blood gas analysis to assess neonatal calf diarrhea and subsequent recovery with an EC-compliant oral electrolyte solution

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Objectives: Electrolyte disturbance, dehydration and metabolic acidosis, accompanied by a strong ion difference (SID), are the most significant consequences of diarrhea in calves. The aim of this observational study was to firstly investigate outbreaks of calf diarrhea on four dairy farms using blood gas analysis. Secondly the study sought to evaluate treatment of diarrheic calves using an oral rehydration and buffering solution (ORBS) that is compliant with current EU legislation.

Materials and Methods: An observational study was conducted on 77 calves (51 healthy, 31 calves with neonatal diarrhea, 5 calves represented in both groups) during outbreaks of diarrhea on four dairy

farms in Ireland. All animals were clinically assessed and scored (CAS) (0= healthy to 4=marked illness). Blood gas analysis was carried out on all animals, with repeated measurements taken in healthy animals, and pre- and post-intervention measurements taken for diarrheic calves. A bench top Rapidpoint 400 (Siemens, Munich, Germany) analyser was used to test all samples. Parameters reported by the analyser included, inter alia, pH, base excess (BE; mM), Na⁺ (mM), K⁺ (mM), Ca²⁺ (mM), Cl⁻ (mM), Glucose (mM), total haemoglobin (Hb; g/dL), standard HCO₃⁻ (mM), and anion gap (AG; mM). For healthy calves, samples were taken over a period of three days, approximately two hours post-feeding. In the case of diarrheic calves, pre-treatment samples were taken within two hours of a milk feed being offered. These calves were then administered an ORBS, 'Vitalife for Calves' (Epsilon Ltd., Cork, Ireland) reconstituted in water according to manufacturer instructions. All treatments were administered by stomach tube.

Statistical analyses were completed in Stata version 12 (StataCorp, USA). Correlations between CAS and blood gas parameters were investigated. Associations between various genetic and environmental factors and blood gas measurements were investigated in the healthy calf cohort. A cross-sectional, time series, generalised estimating equation was used to account for repeated measures.

Results: The mean clinical assessment score of diarrheic calves was 1.7, with 51%, 30%, 17% and 2% of calves scoring 1, 2, 3 and 4, respectively. The mean values for blood pH, BE, AG and SID was 7.26, -4.93 mM, 16.3 mM and 38.59 mM, respectively. The mean clinical assessment score at 6 to 18 hours post-treatment with the ORBS was 0.38 (65% of calves scored 0 and 35% scored 1), which reduced to 0.03 (98% of calves scored 0 and 2% scored 1) within 24 to 48 hours. There was a significant increase in mean values (P<0.001) for pH, BE, HCO₃⁻, Na⁺ and SID after treatment, while a significant decrease was recorded for AG, K⁺, Ca²⁺ and total haemoglobin. The correlation estimates indicate that pH, HCO₃⁻ and BE were strongly and significantly correlated with clinical assessment score, with values exceeding 0.70 in all cases (P<0.05).

Conclusions: Administration of an ORBS formulated on a principle of high SID, coupled to a bicarbonate buffer and supplementary nutritional ingredients, demonstrated rapid recovery from a diarrheic episode in dairy calves. Additionally, we observed measurement of blood pH to be a useful and practical tool in monitoring calf recovery following treatment for diarrhea.

Young Stock

P04-004-154

Effect Of The Oral Administration Of Specific Immunoglobulins Compared To Standard Vaccination On Calf Neonatal Enteritis

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Objectives: Under pressure to reduce the use of antibiotics in animal production, prevention of disease is getting more and more important. Prevention of neonatal calf enteritis (NCE) is commonly performed by vaccination of pregnant dams against the main pathogens involved in NCE.

The objective of the study was to evaluate the clinical effect of an application of specific immunoglobulins concentrate against different strains of E. coli, Rotavirus and Coronavirus given orally to the calves in

addition to normal colostrum on NCE. This procedure was compared to a standard vaccination of the dam against the same pathogens.

Materials and Methods: Calves were chosen in a commercial dairy herd with a standard management and no previous history of high incidence of NCE. This herd had previously not vaccinated against NCE.

For each group of 10 dry cows, half of them were primo-vaccinated with one dose of ROTAVEC® CORONA between 12 and 3 weeks before calving. Each calf born from non-vaccinated cows received one dose of 60ml of LOCATIM® orally within the first 6 hours of life. Every calf received colostrum of his mother.

For each included calf, the farmer registered the vaccination date or the time of LOCATIM® administration, as well as the clinical scoring of diarrhoea during the first 15 days of life. Diarrhoea score was defined according to following criteria: 0 = no diarrhoea; 1 = light diarrhoea without medical treatment; 2 = important diarrhoea requiring oral treatment (rehydration and/or antibiotic therapy); 3 = severe diarrhoea requiring parenteral rehydration or death.

Statistical analyses were performed with the statistical software SYSTAT 12 for Windows®.

Results: 45 (LOCATIM group) and 43 (ROTAVEC group) calves were included in the study. All ROTAVEC cows were vaccinated between 23 and 79 days before calving. All LOCATIM calves received the oral specific immunoglobulins concentrate between 15 and 120 minutes after birth. There was no association between occurrence of diarrhoea and neither time of vaccination before calving nor time of application of the oral specific immunoglobulins concentrate after birth ($p > 0.35$, Kruskal Wallis test or General Linear Model).

The sum of diarrhoea scores was 9 in the LOCATIM group and 29 in the ROTAVEC group. The difference was statistically significant ($p = 0.002$, Mann-Whitney U-test).

18 calves (41.9%) in the ROTAVEC group and 5 calves (11.1%) of the LOCATIM group showed some form of diarrhoea. The difference was statistically significant ($p = 0.001$, Pearson Chi-square test).

If only severe diarrhoea (scores 2 and 3) was considered, 9 / 34 (26.5%) calves were found in the ROTAVEC group vs 3 / 43 (7.0%) in the LOCATIM group. The difference was statistically significant ($p = 0.027$, Fisher's exact test).

Conclusions: In our study, the oral application of specific immunoglobulins concentrate against NCE was effective. Compared to a well-established vaccination against strains of *E. coli*, Rotavirus and Coronavirus, the occurrence and the severity of calf's diarrhoea were significantly reduced.

The oral solution of specific immunoglobulins concentrate warrants the easy and direct transfer of the specific antibodies. It is an effective and simple alternative to vaccination protocols against neonatal calf enteritis.

Comments: In addition, blood samples for the measurement of the specific IgG against *E. coli* F5 (K99), CS31A, F17 (Att25) and F41 as well as against rotavirus and coronavirus were taken. The results of the analyses will be available in April 2016.

Young Stock

P04-004-155

Bovine respiratory disease monitoring in veal calves: clinical signs predictive of calf pneumonia as diagnosed by ultrasonography

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Objectives: A more rational use of antimicrobials for bovine respiratory disease (BRD) would imply only antimicrobial treatment of bacterial infections of the lower respiratory tract and not of the upper airways. Therefore, disease detection systems should monitor symptoms which are predictive of pneumonia and discriminate lower from upper respiratory tract infection. The objective of the present cross-sectional study was to determine which clinical signs are predictive of active pneumonia lesions in 5-8 week old calves, as diagnosed by thoracic ultrasonography.

Materials and Methods: A cross-sectional study was done in two veal farms, determining clinical signs and ultrasonographic findings at the time of a BRD outbreak. Clinical signs were recorded by a single person before the morning feeding. A first set of parameters involved parameters visible from the feeding ally, such as position of head and ears, recumbency, appetite, milk left-overs, nasal and ocular discharge, spontaneous cough and breathing frequency/type. A second set of parameters were collected in a detailed clinical examination (aspect nasal and ocular discharge, ocular mucosae, larynx and trachea reflexes, stridor, sucking reflex, fecal aspect, joint inspection, rectal temperature, heart frequency and trachea + lung auscultation). After recording of the clinical parameters a broncho-alveolar lavage were taken for bacteriological examination. Presence of pneumonia was detected by ultrasonography using a 7.5 MHz linear probe (Mylab One, Esaote Benelux). A calf was considered a pneumonia case when at least one zone of $>1 \text{ cm}^2$ of consolidation was detected. Multivariable logistic regression was used to determine association between the clinical signs and pneumonic lesions and to determine the most sensitive and specific model to predict pneumonia.

Results: Preliminary results on 67 calves are available. In herd 1 40.0% (14/35) and in herd 2 34.4% (11/32) of the calves showed ultrasonographic lesions consistent with pneumonia. In both herds, *Pasteurella multocida* (54.3% in herd 1 and 15.6% in herd 2), *Mannheimia haemolytica sensu lato* (37.1% vs. 6.3%), *Mycoplasma bovis* (42.9% vs. 6.3%) and *Mycoplasma bovirhinis* (22.9% vs. 53.1%) could be isolated from BAL samples. *Histophilus somni* was detected in only 1 calf (herd 1). Clinical signs, univariably associated with pneumonia were breathing frequency ($P=0.05$), lowered head carriage (sign of depression) ($P=0.03$), and pale ocular mucosae ($P=0.04$). The most sensitive final model consisted of breathing frequency (OR= 1.5 (1.1-2.1); $P= 0.02$) for each increase by 10 breaths in a minute) and head position (OR of a lowered head= 3.5 (1.1-11.4); $P= 0.03$). This model had a sensitivity and specificity of 92.7% and 36.0%, respectively and classified 71.2% of the calves correct.

Conclusions: In conclusion, these preliminary results show that few clinical signs can distinguish pneumonia from upper respiratory tract infection in an outbreak situation. Frequently monitored signs such as fever and cough are not among these. A simple model focusing on breathing frequency and head position was most sensitive to detect calves with ultrasonographically visible pneumonia lesions, but resulted in many false positives. Further model building and validation is required to confirm this model.

Young Stock

P04-004-156

Associations between passive immunity and health status for Irish dairy and suckler beef calves

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Objectives: There is a need for information about the passive immune status of calves on commercial dairy and suckler beef farms in Ireland. Hence, the objectives of this study were to: 1) investigate levels of passive immunity in Irish dairy and suckler beef calves and 2) evaluate associations between passive immunity and calf health from birth until 3 months of age.

Materials and Methods: A total of 84 dairy and 111 suckler beef farms throughout Ireland were visited during the autumn 2014 and spring 2015 calving seasons. Blood samples were collected by jugular venipuncture from 1040 dairy and 923 suckler calves between 1 and 21 days of age. Serum was harvested and the Zinc Sulphate Turbidity (ZST) test, which indirectly measures immunoglobulin levels, was performed. ZST results were categorized as follows: LOW (L) = '<10', MEDIUM (M) = '10-20' and HIGH (H) = '>20' units. ZST data were analysed using ordinal logistic regression. Standardised case definitions for disease were provided and farmers were requested to record all disease events. Morbidity was defined as a calf being treated for at least one case of disease between birth and 3 months of age. Morbidity data were obtained for 682 calves from 54 dairy farms and 575 calves from 73 suckler farms. Logistic regression models were constructed to evaluate associations between ZST results and the odds of morbidity.

Results: Mean ZST units for dairy and suckler calves were 18.1 (SD=7.9) and 16.1 (SD=7.4), respectively. Sampling age was significantly associated with ZST status ($P < 0.01$). Dairy calves were less likely to have ZST results in the lower categories than suckler calves (L: 13.9 vs. 21.1%, M: 50.3 vs. 51.0%, H: 35.9 vs. 27.8%, $P < 0.01$). Calves born in spring were more likely to have greater ZST units than autumn-born calves (Dairy – L: 13.0 vs. 19.2%, M: 47.7 vs. 66.7%, 39.3 vs. 14.2%, $P < 0.01$; Suckler – L: 22.8 vs. 16.7%, M: 48.5 vs. 58.1%, H: 28.8 vs. 25.2%, $P = 0.04$). Gender, twin status and colostrum feeding method were not associated with ZST category for dairy or suckler calves. Overall, 16.7% of dairy calves and 25.1% of suckler calves were treated for disease in the first 3 months of life. Calves were most commonly treated for diarrhoea (71.9% of dairy disease events, 29.7% of suckler disease events) or respiratory disease (8.8% of dairy disease events, 25.5% of suckler disease events). The odds of dairy vs. suckler calves being treated for disease were not significantly different (Odds Ratio = 1.6, $P = 0.17$). Suckler calves with LOW ZST units had greater odds of being treated in the first 3 months of life than MEDIUM (Odds Ratio = 2.1, $P = 0.03$) and HIGH ZST suckler calves (Odds Ratio = 2.7, $P = 0.01$). Dairy calves did not significantly differ for morbidity by ZST category ($P = 0.13$).

Conclusions: This is the first large-scale observational study on passive immunity and calf health to be conducted under field conditions in Ireland. These results demonstrate that many Irish calves are at risk of failure of passive transfer of immunity (FPT). A cut-point value of 20 ZST units has traditionally been used to denote FPT. However, recent research has suggested that this cut-point needs to be re-assessed and a value of 11 ZST units was proposed, which would be in line with the LOW ZST category. These results also provide further evidence of a negative relationship between FPT and calf health.

Comments: We acknowledge the DAFM Stimulus Fund (11/S/131) for financial support.

Young Stock

P04-004-157

Monitoring of enteropathogens in young calves in South Belgium : assessment of the diagnostic protocol

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Objectives: The aim of this study is the assessment of the standard protocol for analysis of fecal samples from young calves currently performed by ARSIA (Regional Association for Animal Identification and Health) and the calculation of the prevalence of major enteropathogens of calves in Southern Belgium. The current protocol is designed to find the following pathogens: *Cryptosporidium parvum*, rotavirus, coronavirus and pathogenic enterobacteria such as *Salmonella* spp., *Klebsiella pneumoniae* and the following potentially pathogenic *Escherichia coli* strains : CS31A, F17a, K99 and Enterohemolysin +.

Materials and Methods: Fecal samples of 236 calves under 1 month of age and with diarrhea from 176 Walloon farms have been analyzed between February 2014 and Mai 2015. For each sample, an associated survey provided informations such as the age of the animal at the sampling time.

The standard protocol includes 2 methods of analysis. ELISA were performed for the *Cryptosporidium parvum*, rotavirus and coronavirus detection. Pathogenic enterobacteria are detected by an aerobic culture on Gassner medium. Then, colonies are identified by MALDI-TOF mass spectrometry (Matrix Assisted Laser Desorption Ionisation, Time of Fly). All isolated *E. coli* are serotyped by 2 methods: (1) culture on Enterohemolysine agar (Enterohemolysine +) and (2) culture on MINCA medium following serotyping with 3 antisera (CS31A, F17a, K99).

Data have been analyzed in two ways: a global approach and an age group approach. Then samples have been divided into 6 age categories: 1-3 days (n = 30), 4-6 days (n = 32), 1 week (n = 107), 2 weeks (n = 40), 3 weeks (n = 17) and 4 weeks (n = 10).

Results: Overall results were satisfactory since at least one pathogen was found in 87.29% of the tested samples. However, in the 1-3 days old group, no pathogen was detected in 50% of those. In 51.27% of fecal samples, more than one pathogen was found (up to 70% in the 2 weeks old group). As results are not ranked, this can complicate the interpretation of the data.

Regardless of the calves age, apparent prevalences are 50.42% (95% CI : 44.04 - 56.80) for *Cryptosporidium parvum*, 9.32% (95% CI : 5.61 - 13.03) for coronavirus, 41.53% (95% CI : 35.24 - 47.82) for rotavirus, 36.02% (95% CI : 29.90 - 42.14) for *E. coli* CS31A, 11.44% (95% CI : 7.38 - 15.5) for *E. coli* F17a, 0.85% (95% CI : 0 - 2.02) for *E. coli* K99 and 2.97% (95% CI : 0.80 - 5.14) for *E. coli* Enterohaemolysine +. No *Salmonella* spp. nor *Klebsiella pneumoniae* was found.

Conclusions: This study highlighted the need to improve the standard protocol of young cattle faeces especially for calves of 3 days and under in which, for 50% of them, no pathogen has been found. This can be due to a non-exhaustive research of possible causes, a lack of sensibility of used tests and/or potential treatment effects. When more than one pathogen is found, it would be efficient to rank the results based on the relative importance of each agent. Although *Salmonella* was expected, this bacteria was not detected. Since its prevalence is low, it is likely that the sample size was too small.

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Young Stock

P04-004-158

Association between colostrum, serum natural antibody, hematological parameters, and morbidity in Holstein dairy heifers

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Objectives: The objective of this study was to investigate 3 days old calf serum levels of natural antibodies and its associations with administered colostrum quality (Brix), serum immunoglobulin-G (ELISA), morbidity (incidence of pneumonia, otitis, and diarrhea), mortality, and hematology during the pre-weaning period.

Materials and Methods: Eighty newborn Holstein heifer calves were selected to be part of a prospective cohort study. Colostrum from primiparous cows were pooled and used for the study heifers. All calves were fed 4 L of raw colostrum by esophageal feeder. Colostrum samples for Brix measurements were collected daily 20 minutes before colostrum administration; each calf had a unique Brix measurement relative to the colostrum received.

Twice daily, newborn calves were moved from the newborn pen located in the maternity area to the calf barn. The calf barn was a green-house type of barn, positive ventilated and composed by identical individual-pens (1.5 meter wide by 2 meter long) equally distributed inside the barn. Calves were fed a total of 6 L of pasteurized non-saleable milk divided equally twice a day. Data regarding average daily gain and disease diagnosis were collected by the research group for all study heifers.

Calf pneumonia was defined when two or more of the following clinical signs were detected in a calf: cough, rectal temperature > 39.5°C, respiratory rate > 40 breaths/min, increased cranioventral lung sounds or wheezes. Otitis was defined by observation of ear pain evidenced by head shaking, scratching or rubbing the ears, epiphora, ear droop, and signs of facial nerve paralysis with or without fever > 39.5°C. Following the diagnosis of disease, animals were treated according to the farm protocols.

Blood collection was collected at 3 and 14 days of life. Serum samples were submitted for complete hematology and measurements of IgG and Natural Antibody.

Results: A total of 47 calves born from primiparous and 33 calves born from multiparous were followed from birth until approximately 60 days of life. No significant differences were observed for data collected on calves born from primiparous and multiparous.

A positive correlation was reported between colostrum Brix and serum IgG ($r^2 = 0.45$, $P = 0.001$) and serum NAb ($r^2 = 0.46$, $P = 0.001$) at 3 days of life. Additionally, a very high correlation between serum NAb and serum IgG from 3 days old calves was observed ($r^2 = 0.45$, $P < 0.001$). A critical threshold for serum natural antibody at 3 days of life was determined by a ROC analysis. A threshold value lesser or equal to 0.215 with a 95.24 % sensitivity and 94.92 % specificity detecting calves that had inadequate passive transfer determined by serum immunoglobulin-G (≤ 1000 mg/dl) with an area under the curve (AUC) of 0.986. A significant lower hazard of otitis (HR = 0.55, $P = 0.04$) and CPO calves (HR = 0.45, $P = 0.01$) was reported for calves that had adequate serum levels of natural antibody ($OD_{650} > 0.215$) when compare to calves that did not ($OD_{650} \leq 0.215$).

A receiver-operator characteristic curve was used to determine a critical threshold for Lymphocyte to Monocyte ratio. The cutoff (LMR ≤ 5.67) with a 69.4% Sensitivity and 73.3% Specificity on predicting CPO with an area under the curve (AUC) of 0.718 ($P = 0.008$). A Kaplan Meier survival curve for calves affected with pneumonia and/or otitis was

performed. Calves with low LMR were at 2.53 higher hazard (95%CI: 1.44 – 4.44) of CPO when compared to calves with high LMR.

Conclusions: In this study we suggest a cutoff point of adequate natural antibody levels. Level of serum natural antibody at 3 days of life was significantly associated with colostrum Brix and serum level of IgG. Additionally, calves that had adequate serum level of natural antibody were at a lower hazard of combined events of pneumonia and otitis. There could be more factors in colostrum involved in calf health during the pre-weaning period.

Additionally, for the first time the lymphocyte to monocyte ratio at 14 days of life was reported to be a significant predictor of pneumonia and otitis during the first 60 days of life.

Young Stock

P04-004-159

Implication of Coronavirus in pulmonary lesions in calves

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Objectives: Infectious respiratory disorders in calves are a major problem in dairy and beef herds, with potentially a heavy economic impact. It is a multifactorial problem, and the most common post-mortem diagnosis in calves in first months of age.

Bovine coronavirus (BCoVs) infects the upper and the lower respiratory tract and the intestine. Besides digestive syndromes, BCoVs is known to cause respiratory disease in 2-weeks- to 6-months-old calves and be part of the bovine respiratory complex in feedlot cattle.

The aim of this study is to investigate the implication of BCoVs in pneumonia in Belgian calves on the basis of a case/control study.

Materials and Methods: The study has been performed on a panel of calves standardly autopsied in ARSIA (Regional laboratory specialized in animal health, Ciney, Belgium). The case group includes 115 animals showing respiratory lesions (red and/or grey hepatization, emphysema, abscess, pleurisy, tracheal lesions). The control group involves 49 animals showing no macroscopic pulmonary lesions at necropsy. Calves were aged from 1 week to 6 months.

For each calf, lung was sampled and submitted to various tests : (I) culture on classical media, (II) antigen ELISA for detection of Mycoplasma bovis, Bovine Respiratory Syncytial Virus (BRSV) and Parainfluenza-3 virus (PI-3) (Bio-X), (III) real-time PCR for detection of Coronavirus (LSI VetMAX™ Triplex Ruminant Rotavirus-Coronavirus, Thermo Fisher Scientific) and other pathogens (Mycoplasma bovis, Histophilus somni, Pasteurella multocida, Mannheimia haemolytica, BRSV, Bovine Viral Diarrhea virus, Bovine Herpesvirus type 4 – Thermo Fisher Scientific kits). Any real-time PCR result was considered positive if the cycle threshold (Ct) was inferior to 45; any result with non-conforming internal positive control was excluded from data analysis.

A histopathological study was led on 59 lungs (40 with macroscopic lesions, 19 without).

Faeces of calves were tested with bovine Coronavirus ELISA kit (Bio-X).

Results: About 24,55% (27/146) of the tested calves (displaying or not macroscopic lesions) were shedding BCoVs in their lungs upon

real-time PCR results. There was no significant difference between prevalences in case (21,69%) and control (33,33%) groups (Chi-square test : $p > 0,20$). Ct values of the positive results were relatively high (mean = 34,95). When lowering the positivity threshold of real-time PCR test at 30, no positive result was present in the control population and only one positive result (Ct,27,39) was seen in calves showing respiratory lesions (1/146, soit 0,68%). At necropsy, this 22 days old calf was exhibiting red hepatization of the lung. No other respiratory pathogens could be detected in the lung. Diarrhea was also reported but without BCoV detected in faeces.

In calves showing lesions and harbouring BCOVs, the virus was associated with one or more other respiratory pathogens in 96,77% of the cases. There was no significant association between BCOVs and other respiratory pathogens.

On the basis of the histopathological examination, there was no significant difference (Fisher's exact test: $p = 0,63$) in percentages of positivity between calves showing microscopic lesions with a viral pattern in the lungs (18,18%) and calves without viral inflammatory lesions (12,5%).

There was no correlation between BCOVs detection in faeces and its detection in lungs ($\kappa = 0,018$).

Conclusions: In the context of this case/control study, BCOVs, alone, doesn't seem to be a major pathogenic agent in calf pneumonia in Belgium. A large percentage of calves were harbouring BCOVs in lower respiratory tract without pulmonary disease. The exact role of this virus in bovine respiratory disease is not fully understood though it is known that co-infection with different pathogens, environmental factors and/or host health influence its implication.

Young Stock

P04-004-160

Metabolomic analysis of calf serum after being fed colostrum

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Objectives: Calves obtain nutrients and immunoglobulins (Ig) from colostrum. The amount of IgG increases after the intake of colostrum until 24 h after birth. Since this increase in IgG is accompanied by increased levels of other milk proteins in serum, we hypothesize that there may exist mechanisms for the non-specific absorption of intact proteins without degradation in the intestine. If this hypothesis is valid, the serum levels of low-molecular weight compounds derived from colostrum should increase after the intake. Here we examined temporal changes in the concentrations of low-molecular weight compounds in serum from calves that were fed colostrum.

Materials and Methods: Serum samples were collected from five male Holstein-Friesian calves for 12 h after being initially fed colostrum of 4% body weight at once by themselves within 6 h after birth. Serum proteins were analyzed by SDS-PAGE under non-reducing conditions. Acetone extracts of their serum samples were dried using a spin drier and derivatized with MSTFA. The samples were analyzed in triplicate using two dimensional gas chromatography (GC)-time of flight (TOF)-mass spectrometry (MS) (Pegasus 4D, LECO). ChromaTOF software (LECO) with analyte detectability and statistical comparisons was used to evaluate the differences between sample groups. Statistical analysis software was used for the multi-variable analyses, including principal

component analysis and cluster analysis, in order to identify organic compounds undergoing in temporal changes in concentration after colostrum feeding.

Results: SDS-PAGE showed a drastic change in a coomassie brilliant blue (CBB) stained band corresponding to IgG after feeding with colostrum. In addition to temporal changes in IgG levels, there were only a few changes visible in GC-MS total ion chromatograms after feeding. However about 2500 peaks were identified from each sample and the variation between samples aligned to over 4000 peaks. Fisher ratio of 100 narrowed these peaks to about 300 peaks. Several compounds in the serum, categorized into sugars, amino acids, and fatty acids, were identified and varied before and after feeding. Temporal changes in the concentrations of some of these compounds correlated with that of IgG.

Conclusions: This study demonstrates that changes occur in the metabolomic patterns of low-molecular weight organic compounds in calf serum after being fed colostrum. These changes do not correlate with protein absorption. We propose that proteins are not non-specifically absorbed along with low-molecular weight organic compounds, but are rather absorbed selectively via the intestinal epithelium of neonatal calves.

Young Stock

P04-004-163

Potential benefits of acidification when feeding waste milk to calves

Roger Blowey^{1,1}; Jack Griffiths¹

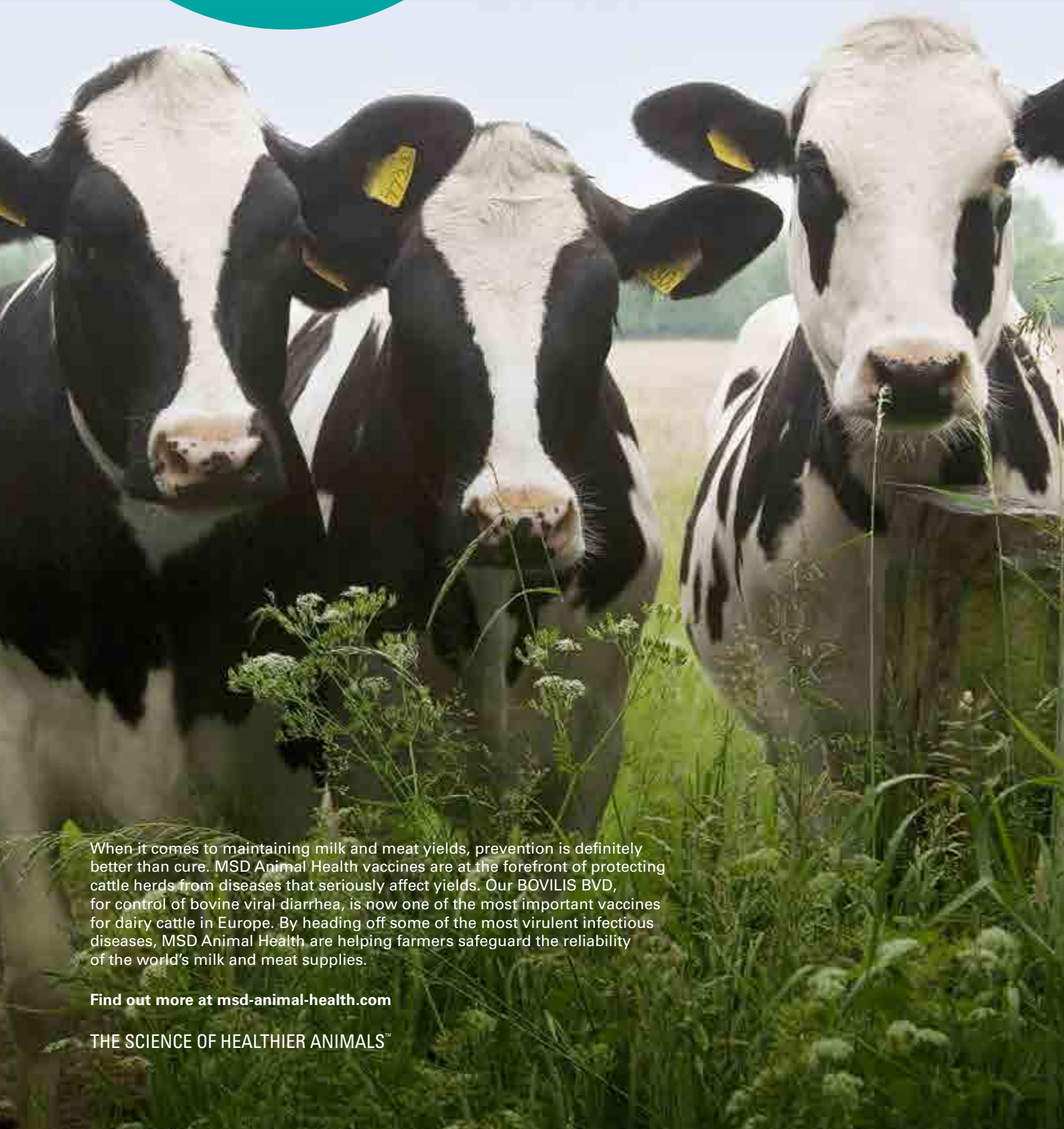
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Objectives: In the UK waste milk is defined as milk from cows in the first few days of lactation; milk from cows under treatment, milk from cows with mastitis and other conditions requiring a drug withhold period. The risks of feeding of waste milk include a high bacterial count leading to digestive upsets; the potential spread of Johnes (MAP), TB, and Mycoplasma; possibly increasing the risk of mastitis when they are heifers; and the potential risk of antibiotic resistance. Many of the risks can be inexpensively reduced or eliminated by acidification with formic acid, the effectiveness of which was the objective of the current study.

Materials and Methods: In a Johnes positive 600 cow dairy herd rearing its own replacements, heifer calves were originally fed calf milk replacer as a Johnes control measure, the waste milk going to male calves reared for beef. However it was noted that growth rates and disease levels were better in the male calves. Since 2009 acidified waste milk has been fed to all calves, including waste milk from Johnes positive cows. Formic acid is diluted 1:10 parts of water, then 30ml is added to one litre of milk to reach a pH of 4.0 and the milk left to stand for 48 hours with occasional stirring as described by Anderson (2008). When these calves enter the dairy herd as cows they are serological screened and ELISA tested at the end of each lactation. Positive cows are given a red ear tag, served with beef semen and culled early.

Results: The prevalence of seropositive cows originating from calves ($n = 311$), fed milk replacer was compared to cows originating from calves ($n = 134$) fed on purely acidified waste milk. The apparent prevalence of Johnes's positive cows was 13.83% with milk replacer (mean parity 3.17) compared to 1.50% prevalence with acidified milk (mean parity 1.66). The difference was statistically significant to a 95% confidence level with a p-value of 0.002. In addition there was a 26% reduction in the number of calves treated for pneumonia, and a reduction in the number of calves

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treated for diarrhoea from 18% of calves treated in 2008/2009 to 6% in 2010/2011. Estimates of waste milk are estimated to be 1.5% of total output, eg 1.5% of 7000 litres = 100 litres/cow/year, plus discard from the first 6 milkings, eg 25 litres/day for 3 days = 75 litres. Waste milk is also of higher quality than calf milk replacer. The financial and environmental savings are therefore considerable

Conclusions: Although this study shows a reduction in the apparent prevalence of Johnes, it should be noted that the mean age of the milk replacer group (3.17) was higher than the acid milk group (1.66), and it would be expected that the prevalence of ELISA positive animals would increase with increasing age. There was also no direct control group, i.e. no animals fed milk replacer at the same time as the acid milk. However the results do show that there is no evidence of any increase in Johnes positive cows despite waste milk from Johnes positive animals being fed to calves.

Young Stock

P04-004-164

Study of main enteropathogens associated with *Cryptosporidium* during diarrhea in calves

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Objectives: *Cryptosporidium* is one of the main etiologies of diarrhea in newborn calves, mixed infections are commonly found. The aim of this study was to evaluate the main enteropathogens associated with *Cryptosporidium* spp. in newborn calves with diarrhea under different feeding strategies.

Materials and Methods: A total of 17 newborn male calves Holstein divided into two groups, 8 calves in group 1, fed 4 liters of milk replacer per day and 9 calves in group 2, receiving 6 liters. From the first day of life, the feces of calves were evaluated daily after breastfeeding in the morning and classified as diarrhea or no diarrhea. The first day of diarrhea until the seventh, feces were collected on alternate days (1, 3, 5 and 7 days). Qualitative research of oocytes of *Cryptosporidium* spp was performed by centrifugal sedimentation method in water-ether and modified Ziehl-Neelsen staining; qualitative research of *Giardia* sp. through concentration by centrifugal flotation in saturated solution of sucrose; isolation and PCR to identification of *Salmonella* spp. and pathotypes of *E. coli* and identification of Rotavirus by standard technique of polyacrylamide gel electrophoresis.

Results: All calves had diarrhea during the collection period. The average age of the beginning of the observed diarrhea in group 1 and 2 was 5.87 and 5.11 days of life, respectively. The association *Cryptosporidium* spp. with other agents was observed in 62% of positive samples, and these, 41% were associated with *E. coli* pathotypes, 35% with *Salmonella* spp., 18% with *Giardia* and 6% with Rotavirus in group 1. In Group 2, it was found 71% associated with *Salmonella* spp., 21% with *Giardia* sp. and 7% with Rotavirus.

Conclusions: Although the literature demonstrate Rotavirus as the most common agent associated with *Cryptosporidium* spp., the association between *Cryptosporidium* spp. and bacteria was the most common in both groups, such fact may be related to environmental conditions conducive to maintaining high bacterial load.

Young Stock

P04-004-165

Evaluation of factors affecting overall colostrum quality and passive transfer of immunity to calves using a Brix refractometer

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Objectives: Brix refractometers are inexpensive, easy to use and quick cow-side tools for the indirect assessment of colostrum quality (CQ) and passive transfer of immunity (PTI); however, factors affecting the latter have not been evaluated in large scale studies using this method. The objective of the study was the evaluation of factors affecting overall CQ and passive transfer of immunity to newborn calves using a Brix refractometer (BR).

Materials and Methods: The study was conducted in 9 commercial dairy herds in Northern Greece from February to October 2015. 560 Holstein cows and 510 calves were used. Each cow was blood sampled the 1st day post calving and the percentage (%) of serum total protein (sTP) was measured within 3 hours using a digital BR. Cows were milked completely 2-8 hours after calving, colostrum quantity recorded and 2 samples were collected from each cow: one was used to measure % of total solids cow-side, using the same BR; the 2nd sample was transferred to the laboratory and analyzed for fat, protein and carbohydrate content with the use of Milkoscan. Quantity of 1st colostrum meal fed to each calf and time interval between calving and 1st meal was recorded. Calves were blood sampled between 24-48 hours post-calving and % of sTP was measured within 3 hours using the BR. Data of colostrum quantity were analyzed with a univariate general linear model (UGLM); season and time interval between calving and colostrum collection were set as fixed effects. Brix measurements were analyzed with UGLM. For colostrum, the model included season, time interval between calving and colostrum collection, quantity of colostrum produced and cows' sTP as fixed effects. For calf serum it included, season, colostrum quantity and quality fed, time interval between calving and 1st colostrum meal and cows' sTP as fixed effects. Correlations between colostrum fat, protein and carbohydrate content, and Brix scores for colostrum and cow serum measurements were estimated with bivariate analysis (correlation coefficient by Pearson).

Results: The average Brix scores for colostrum, cow serum and calf serum were 25.5±4.5%, 9.5±0.7% and 8.8±1.2%, respectively. Season and time interval between calving and colostrum collection had a significant effect on colostrum quantity produced (P<0.05); the latter being lowest in autumn and when time interval was short. Season, time interval between calving and colostrum collection and quantity of produced colostrum had a significant effect on CQ (P<0.05). The latter was higher in spring, when the interval between calving and colostrum collection was short and when the quantity produced was low. The effect of cow sTP content on colostrum quality was not significant (P>0.05). Colostrum quantity and CQ fed had a significant effect (P<0.05) on calf serum Brix scores, with highest quality and quantity resulting in better PTI. The effect of season, time interval between calving and 1st colostrum meal and cow sTP content were not significant (P>0.05) for PTI. Colostrum fat and protein content were negatively correlated (P<0.05) with quantity produced (r=-0.105 and r=-0.211, respectively), but positively correlated (P<0.05) with Brix scores (r=0.173 and r=0.926, respectively). Colostrum carbohydrate content had a statistically significant (P<0.05) positive correlation with

colostrum quantity produced ($r=0.109$) and a statistically significant negative correlation with Brix colostrum measurement ($r=-0.434$). Colostrum protein content was also positively correlated with total protein measured by Brix in cow serum ($r=0.103$).

Conclusions: Factors known to affect CQ and PTI were confirmed using Brix refractometer scores. In calves, CQ and quantity fed had a significant effect on PTI. In cows, serum total proteins affect colostrum protein content but neither colostrum quality nor PTI, as assessed by Brix refractometry.

Young Stock

P04-004-166

Longitudinal study of naturally occurring rotavirus diarrhea in a vaccinated and a non-vaccinated dairy herd in Sao Paulo State - Brazil

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Objectives: The aim of the present study was to evaluate diarrhea characteristics and identify VP7 (G) and VP4 (P) genotypes of bovine group A rotavirus (BRV-A) from a vaccinated and a non-vaccinated dairy herd from Sao Paulo State, Brazil.

Materials and Methods: Animals belonging to a herd that regularly vaccinated the dams according to manufacturer's instructions with a commercial vaccine containing strains G6P[1] and G10P[11] of inactivated BRV-A (group V), and cows from a herd that was not regularly vaccinated against rotavirus diarrhea (group NV) were evaluated in a longitudinal design study. Colostrum management consisted of bottle feeding at least 4 liters of their own mother's colostrum to calves up to two hours after birth. Fifty six Holstein calves, 28 from group V, and 28 from group NV were evaluated from birth to 30 days of age. Fecal samples were taken directly from rectum using individual plastic bags before colostrum intake (0), and with 1, 2, 7, 15, 21, and 30 days of age and frozen at -20°C until analysis. Whenever calves presented diarrhea, fecal samples were taken at 24 hour intervals until the end of clinical signs. Fecal samples were classified in scores: 0 (firm feces), 1 (slightly loose feces), 2 (loose feces), and 3 (watery feces). Scores 0 and 1 were considered normal feces and scores 2 and 3 were considered diarrhea. Rotavirus detection in feces was performed using 7.5% polyacrylamide gel electrophoresis technique. The polymerase chain reaction (PCR) preceded by reverse-transcription (RT) of viral RNA was employed to characterize bovine rotavirus genotypes G (VP7) and P (VP4) using primers already reported. First round PCR products were subjected to bidirectional sequencing and analyzed with Neighbor-Joining algorithm, Maximum Composite Likelihood as substitution model, and 1,000 bootstrap replicates.

Results: A total of 561 fecal samples were taken from calves from birth to 30 days of age, 281 from group V, and 280 from group NV. In group V, 211 (75.1%) fecal samples were considered normal (scores 0 and 1) and 70 (24.9%) fecal samples were considered diarrheic. In group NV 212 (75.7%) fecal samples were considered normal and 68 (24.2%) were considered diarrheic. Group V comprised eleven samples originating from six different calves that tested positive

for BRV-A detection in feces by PAGE technique; while group NV comprised three samples originating from three different calves that tested positive for BRV-A. Although positive samples were found in both normal ($n=5$) and diarrheic feces ($n=9$), all calves that tested positive for BRV-A detection in feces presented an episode of diarrhea in the first 30 days of life. Bovine RVAs analyzed in this study showed G/P-genotypes of G6P[11] from group V and G6P[5] from group NV. Regarding calves age, none of the calves that tested positive for BRV-A in feces were aged between 1 and 7 days of life. Calves that tested positive rotavirus in feces were in their second ($n=2$) or third weeks of age ($n=4$) in group V and in their second ($n=2$) and fourth ($n=1$) weeks of life in group NV.

Conclusions: All calves infected by rotavirus presented diarrhea episode in the first month of life. Vaccination of herds may induce genetic reassortment of the virus, as seen by the genotype of rotavirus found in the calves born to vaccinated dams. Commercial vaccines currently used are not designed for the prevention of rotavirus infection, but rather to decrease morbidity, severity of diarrhea and mortality in field conditions, which, does not reduce the expenses of treatment aiming to avoid secondary bacterial infections and still impacts on the potential development and performance of calves in their productive life.

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Epidemiology

P03-003-084

Chronic herd health problems in Germany: Description of symptoms on herd level and risk factor analyses

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Objectives: In the last two decades, chronic herd health problems in dairy herds accompanied by economic losses and a compromised animal welfare have been reported in Germany. First, a causal relationship with *Clostridium botulinum* was suggested. However, *C. botulinum* could not be proven to be the main exposition factor. Therefore, the causes of the ongoing herd health problems remain unclear. Hence, the objectives of this presentation are (I) to describe the clinical symptoms systematically on herd level and (II) to identify risk factors from various fields including housing, hygiene, feeding, infectious diseases and claw care.

Materials and Methods: To examine the herd health problems, a case-control study was conducted. Due to the unspecific clinical picture, case-farms had to fulfill at least three of the following five criteria: decreased milk yield, increased mortality, increased culling rate, increased number of downer cows and the farmers' impression of herd health problems. Control farms (n=47) did not fulfill any of these criteria. Case 1-farms (C1, no vaccination against any Clostridia; n=45) and case 2-farms (C2, multi-clostridia-vaccination was applied; n=47) were compared to control-farms, separately. During a single farm visit, trained study veterinarians scored the herd with regard to cow-comfort, locomotion, body condition, hygiene, and skin lesions. Moreover, an interview was held with the herd manager, housing conditions and feeds were assessed and ten cows were examined thoroughly.

Results: A combination of symptoms from all analyzed fields except udder health showed a significant relation to the case-control status. Multifactorial logistic regression modeling revealed that case-farms had significantly fewer cows with high body condition scores (C1: $p=0.0102$, C2: $p=0.0041$), more cows with skin lesions on legs (C1: $p=0.0400$, C2: $p=0.0054$), and a lower cow-comfort-quotient than control-farms (C1: $p=0.0095$, C2: $p=0.0048$). Moreover, case 1-farms had a longer calving interval ($p=0.0120$), and more often a deficient cow hygiene ($p=0.0419$) than control farms. Case 2-farms had a lower cud-chewing-index than control-farms ($p=0.0282$).

Concerning the risk factor analyses, both types of case farms had a lower energy density in the roughage for fresh lactating cows (C1: $p=0.0088$, C2: $p=0.0052$). Case 1-farms had more often lying areas in worse hygienic conditions ($p=0.0114$) and were more likely to have an intermediate animal-watering place-ratio ($p=0.0303$) compared to control farms. Case 2-farms had more often raised cubicles instead of cubicles with deep beddings or straw yards ($p=0.0031$), were more likely to have at least one silage with microbiological deviations ($p=0.0170$), and had smaller herds (confounder, $p=0.0290$) compared to control farms. Differently than expected, case 2-farms were less likely to have at least one grass silage with a true protein fraction <50% of crude protein ($p=0.0112$) and performed herd claw trimming more frequently twice a year ($p=0.0220$). Perhaps, farmers had already increased claw trimming frequency due to the high incidence of lameness.

Conclusions: The detected symptoms confirm that herd health problems were unspecific but yielded important knowledge about the problems case-farms had. This knowledge should be used in the future to adjust consultancy to these well-known but still relevant problems.

It can be concluded that feeding (in particular the energy density and the quality of silages), resting areas and hygiene presumably play an important role. It does not seem that there is the one and only factor responsible for chronic herd health problems in Northern Germany. However, we conclude that no field should be neglected when herd health problems have to be solved.

Abortion/Stillbirth Investigation

P04-004-004

Evidence of exposure to pathogens in stillborn calves on Polish dairy farms - preliminary results

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Objectives: Calves born dead after a gestation period of ≥260 days or within 24 hours of birth may be considered stillborn (SB). The causes of SB are multifactorial and may differ between herds and between countries internationally. Numerous infectious causes of bovine stillbirth have been documented. Compared to abortion (birth of a non-independently viable fetus pre-term) infection is less commonly diagnosed in SB calves. There are no published data regarding the occurrence of intrauterine infection in SB calves in dairy cattle in Poland. Therefore the aim of this study was to investigate the prevalence of intrauterine infections in stillborn calves.

Materials and Methods: The study was carried out on 121 SB calves (as defined above), born from November 2013 to June 2015 in 30 Polish Holstein-Friesian herds (1-1,031 cows/herd; 1-35 SB/herd). None of the calves received colostrum. A complete necropsy was performed on each carcass. During the necropsy an ear sample, blood for plasma, nine internal organs (spleen, liver, lung, small intestine, kidney, adrenal glands, heart, brain) and abomasal contents were sampled. The placenta was also sampled. Samples from all internal organs and the placentae were cultured in aerobic (for routine culture and *Salmonella spp.*), anaerobic and microaerobic conditions. The ear samples were tested for Bovine viral diarrhoea virus (BVDV) antigen (ELISA). Sections of inner organs were tested using real time PCR to detect *Neospora caninum*, Schmallenberg virus (SBV) in brain, Bovine herpesvirus type 1 (BHV-1) in liver and pathogenic *Leptospira spp.* in kidney. Additionally, sections of brain were examined histologically. Foetal blood plasma was tested for antibodies to *Neospora caninum*, BHV-1, BVDV, SBV (ELISA) and *Leptospira hardjo* and *L. pomona* (microagglutination test).

Results: No primary bacteria were cultured from the internal organs, abomasal contents or placentae. In two calves, an abundant growth of *E. coli* (1 case) or *Enterococcus faecalis* (1 case) from all internal organs was observed. One calf was positive for BVDV (ear biopsies). All real-time PCR results were negative. Antibodies were detected to: BVDV (4 cases), *N. caninum* (5 cases), SBV (5 cases), *L. hardjo* (1:100-3 cases, 1:400-1 calf), *L. pomona* (1:100-1 case, which had antibody for *L. hardjo* as well). Antibodies to BHV-1 were not detected. Of the 90 brains examined *N. caninum* cysts were detected in 7 calves (one of which also had antibodies to *N. caninum*).

Conclusions: This study demonstrated that previous exposure to pathogenic infectious agents, as evidenced by the detection of a humoral immune response, was uncommon (~15% of SB) and that evidence of infection was even less common (~6% of SB) in Polish dairy herds.

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Abortion/Stillbirth Investigation

P04-004-007

Differentiation of acute intrauterine infection from other causes of death in bovine perinatal mortality using SAA and IgM concentrations.

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Objectives: Bovine stillbirth may be defined as calf death at full term (>260 days) prior to, during, or within 48 h of calving. The unexplained stillbirth (approximately 30% of all deaths) is a diagnostic challenge for veterinary practitioners and for laboratory diagnosticians. The high rate of unexplained stillbirths in calves may, in part, be the consequence of too narrow a spectrum of diagnostic methods applied. The objective of this study was to determine diagnostic threshold for immuno-inflammatory biomarkers in stillborn calves diagnosed with intrauterine infection (bacterial, viral or parasitological) compared to traumotocia and unexplained cases.

Materials and Methods: The 110 calves were from perinatal mortality necropsy cases from Irish dairy farms in 2011-2013. These were full-term (>260 days of gestation), died within one hour of birth but did not consume colostrum. Four diagnostic groups were compared: acute intrauterine infection (INF_A; n=16), chronic intrauterine infection (INF_CH; n=31), traumotocia (TRAUM; n=22) and unexplained death (UNEXPL; n=41). In the INF_A group there was detection of: *Bacillus licheniformis* (5 cases), *Salmonella Dublin* (7 cases), *Trueperella pyogenes* (3 cases) or *Listeria monocytogenes* (1 case). In the INF_CH group there was: BVD virus (7 cases), *Neospora caninum* (6 cases) or Schmallenberg virus foetopathy (18 cases). In the TRAUM group there were detection of severe antemortem lesions consistent with iatrogenic parturient trauma at assisted calvings. The case inclusion criteria for the UNEXPL group were failure to fulfill the inclusion criteria of the other three groups.

Plasma was analyzed for serum amyloid A (SAA), (ELISA), haptoglobin (Hp), (microplate guaiacol method or ELISA) and immunoglobulins M (IgM) and G1 (IgG1), (both ELISA).

A logistic regression model –was used to evaluate which parameter - SAA, Hp, IgM and IgG1 would discriminate acute infection from other COD categories. An ROC analysis was used to evaluate the ability of parameters to discriminate between acute infection and other COD.

Results: The mean (sem) concentrations of SAA (mg/L), and IgM (mg/L) were numerically higher in the INF_A [SAA 53.3 (16.5), IgM 370.3 (144.7)] compared to the INF_CH [SAA 19.6 (10.1), IgM 86.1 (31.0)], TRAUM [SAA 11.9 (2.4), IgM 81.5 (40.4)] and UNEXPL groups [SAA 10.7 (3.0), IgM 134.2 (49.2)]. SAA concentrations in INF_A group were significantly higher than in UN group ($P=0.04$) and tended to be significantly higher than in INF_CH ($P=0.06$). IgM tended to be significantly higher in INF_A compared to TRAUM ($P=0.051$) and compared to INF_CH ($P=0.09$). Hp and IgG1 values were not significantly different ($P>0.05$) between the groups. The logistic regression model showed that SAA and IgM, but not Hp and IgG1, values could significantly differentiate acute infection from other COD. The area under curve for SAA was 0.705 (95% CI 0.533-0.877, $P=0.02$), and IgM 0.719 (95% CI 0.579-0.858, $P=0.002$). The optimal threshold for diagnosis of acute infection in stillborn calves was ≥29.5mg/L for SAA (Sensitivity=0.56, Specificity=0.91) and ≥52.1 mg/l for IgM (Sensitivity=0.75, Specificity=0.68).

Conclusions: The SAA and IgM concentrations were higher in stillborn calves with acute infection compared to other causes of death. The concentration of SAA and IgM at/above diagnostic thresholds with highest combined sensitivity and specificity may be useful in detecting bacterial infections in stillborn calves. This is the first report which shows that these immune-inflammatory biomarkers can differentiate acute intrauterine infection from others causes of death.



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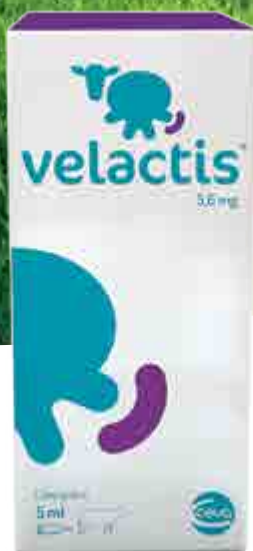
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